U.PORTO

FACULDADE DE CIÊNCIAS
UNIVERSIDADE DO PORTO

INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR
UNIVERSIDADE DO PORTO

Synthesis of precursors of new analogs of the vitamin D changed in the bicyclic ring CD

Sandra Filipa Pires da Silva

Mestrado em Bioquímica

Faculdade de Ciências da Universidade do Porto

2019

Orientador

José Enrique Rodríguez Borges, Faculdade de Ciências da Universidade do Porto

Coorientador

António Mouriño Mosquera, Facultad de quimica da Universidad de Santiago de Compostela

Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, _____/_____/_____

Agradecimentos

Antes de mais quero agradecer ao programa Erasmus + por me ter dado a oportunidade de fazer esta tese em colaboração com o departamento de química orgânica da faculdade de química da Universidade de Santiago de Compostela.

Ainda relacionado com este programa quero agradecer às relações internacionais da Universidade do Porto pelo acompanhamento da minha estadia na Espanha e por todo o apoio demonstrado.

Quero agradecer aos investigadores que trabalham nos laboratórios quer em Portugal quer na Espanha por me terem guiado e ajudado. Em especial ao Xiao e ao Ivo por me terem acompanhado durante a tese,

Quero agradecer também a todos os meu amigos e todas as pessoas que conheci durante este ano por me terem dado força e muito apoio que foram essenciais para poder ter terminado esta tese.

Em especial quero agradecer aos anjinhos que acompanharam este meu percurso, a Carolina e a Isabel e à minha família por todo o apoio e carinho, sem vocês não teria conseguido terminar este ciclo.

Quero também agradecer à Dr. Adelaide Teles por todo o apoio prestado, foi mesmo muito importante.

Por último agradecer ao meu orientador, professor Enrique Borges, e ao meu co-orientador, professor António Mouriño, pois foram quem tornou toda esta tese possível.

Agradecer ainda o financiamento dado pelo Estado Português através da FCT – Fundação para a Ciência e a Tecnologia no âmbito do projeto com a referência PTDC/BIAMIB/29059/2017 - Síntese e Avaliação Biológica de Sila-Secoesteroides, financiado por Fundos FEDER através do Programa Operacional Competitividade e Internacionalização - COMPETE 2020 (POCI-01- 0145-FEDER-029059).

Abstract

In the last years there have been studies about the therapeutical effects of calcitriol. The use of this compound in therapeutical quantities has been associated with calcemic effects which has been made infeasible the use of this molecule as a therapeutic drug. Due to these secondary effects the investigation of new analogs has increased.

Therefore, the objective of this project is the development of new calcitriol analogs.

Docking studies showed some promising values to three analogs, which are the final molecules that we wanted to synthesize. In this work has been tried the synthesis of the CD ring precursors of these analogs using two different methods. One of the methods was done in Porto and the other one in Spain.

The three analogs that we proposed to synthetize are enantiomerically different from the calcitriol molecule, and so, one of the main difficulties in their synthesis is obtaining the enantiomerically pure CD ring precursors. Consequently, in this thesis we had focused in resolution strategies, like esterification with a chiral molecule, to obtain the corresponding diastereomeres, the use of enzymes as a catalyst of this esterification, and the asymmetric Sharpless epoxidation.

Most of these strategies did not worked and so, it is necessary more studies in order to obtain the wanted analogs.

Key words

Vitamin D, Calcitriol analogs, CD bicyclic ring, Wittig reaction, ylide, Alcohol resolution, Sharpless asymmetric epoxidation

Resumo

Nos últimos anos tem-se investigado os efeitos terapêuticos do calcitriol. A utilização deste composto em quantidades terapêuticas tem sido associada a efeitos calcémicos que inviabilizam o uso desta molécula como fármaco. Devido a esses efeitos secundários, a procura de novos análogos tem aumentado.

Portanto, o objetivo deste projeto é o desenvolvimento de novos análogos de calcitriol.

Os estudos de *Docking* feitos mostraram alguns valores promissores para três análogos, que são as moléculas finais que se pretende sintetizar. Neste trabalho tentou-se fazer a síntese dos precursores do biciclo CD desses análogos usando dois métodos diferentes. Um dos métodos foi realizado no Porto e o outro na Espanha.

Os três análogos que propusemos sintetizar são enantiomericamente diferentes da molécula do calcitriol e, portanto, uma das principais dificuldades na sua síntese é a obtenção dos precursores do biciclo CD enantiomericamente puros. Portanto nesta tese o foco foi o estudo de estratégias de resolução, como a esterificação com uma molécula quiral, para obter os diastereoisomeros correspondentes, o uso de enzimas como catalisadores dessa esterificação e a epoxidação assimétrica de Sharpless.

A maioria destas estratégias não funcionou e, portanto, são necessários mais estudos para obter os análogos desejados.

Palavras Chave

Vitamina D, Análogos do Calcitriol, Biciclo CD, Reação de Wittig, Ileto, Resolução de álcoois, Epoxidação assimétrica de Sharpless

Index

Agradecimentos iii
Abstract v
Resumo vi
Index vii
Scheme index xi
Figure index xiii
Abbreviations xiv
Compounds index xvi
Introduction 1
1. Vitamin D 1
1.1. Vitamin D chronology 1
1.2. Vitamin D system 2
1.3. Function and biological activity of calcitriol 4
1.3.1. VDR Receptor 4
1.3.1.1. DNA binding domain 4
1.3.1.2. Ligand binding domain 5
1.3.2. Calcitriol mechanisms of action 6
1.3.2.1. Genomic mechanism 6
1.3.2.2. Nongenomic mechanism 7
1.3.3. Biological activity 8
1.4. Diseases and therapeutical applications 8
2. Calcitriol analogs 9
2.1. Structure function relationship of vitamin D 9
2.1.1. Conjugated triene (seco-B ring) and A ring 9
2.1.2. C/D ring 10
2.1.3. Side chain 10
2.2. Analogs with clinical interest 11

2.3. Synthesis of calcitriol 12
2.3.1. Synthesis of triene unit and A ring 12
2.3.2. Enantioselective syntheses of the CD-ring 17
3. Bioavailability 19
Objectives 21
Results discussion 22
1. Docking 22
1.1. Docking results for the calcitriol analog A 23
1.2. Docking results for the calcitriol analog B 23
1.3. Docking results for the calcitriol analog C 24
1.4. Analogs results comparison 25
2. Strategy and synthetic plan 26
3. Work done in Porto 30
3.1. Synthesis of the alcohols 4 and 17 30
3.2. Synthesis of the alcohols 6 and 19 32
3.3. Synthesis of the protected alcohols 7 and 20 33
3.4. One of the strategies used for the resolution of the alcohol 7 35
3.4.1. Synthesis of the ketone 9 35
3.5. Deprotection of alcohols 6 and 19 37
3.6. Chiral resolution 38
3.6.1. Resolution using resolving agents 38
3.6.2. Resolution method using different enzymes 40
3.7. Resolution method using a Sharpless asymmetric epoxidation 44
4. Work done in Spain 46
4.1. Synthesis of the alcohols 4 and 17 46
4.2. Synthesis of the ester 11 46
4.2.1. TEMPO oxidation 47
4.2.2. Wittig reaction 48

4.2.2.1. Phosphorous ylide synthesis (Y) 49
4.3. Reduction to obtain the alcohol 12 51
Discussion and conclusions 53
Future perspectives 54
Experimental procedure 55
1. Materials and methods 55
2. Work done in Porto 57
2.1. Synthesis of Ylide 57
2.2. Bicyclic ring 6,5 58
2.2.1. Synthesis of 2-(hex-5-yn-1-yloxy) tetrahydro-2H pyran (**2**) 58
2.2.2. Synthesis of benzyldimethyl(6-((tetrahydro-2H-pyran-2-yl) oxy) hex-1-yn-1-yl) silane (**3**) 58
2.2.3. Synthesis of 6-(benzyldimethylsilyl)hex-5-yn-1-ol (**4**) 59
2.2.4. Synthesis of 6-(benzyldimethylsilyl) hex-5-ynal (**5**) 60
2.2.5. Synthesis of 8-(benzyldimethylsilyl)oct-1-en-7-yn-3-ol (**6**) 60
2.2.6. Synthesis of benzyl(6-((tert-butyldimethylsilyl) oxy)oct-7-en-1-yn-1-yl)dimethylsilane (**7**) 61
2.2.7. Synthesis of 8-(benzyldimethylsilyl)oct-1-en-7-yn-3-ol (**6**) 62
2.2.8. Synthesis of 3-(benzyldimethylsilyl)-7-((tert-butyldimethylsilyl)oxy)-1,4,5,6,7,7a-hexahydro-2H-inden-2-one (**9**) 63
2.2.9. Resolution attempt 1 64
2.3. Bicyclic ring 5,5 64
2.3.1. Synthesis of 2-(pent-4-yn-1-yloxy)tetrahydro-2H-pyran (**15**) 64
2.3.2. Synthesis of benzyldimethyl(5-((tetrahydro-2H-pyran-2-yl) oxy)pent-1-yn-1-yl) silane (**16**) 65
2.3.3. Synthesis of 5-(benzyldimethylsilyl)pent-4-yn-1-ol (**17**) 65
2.3.4. Synthesis of 5-(benzyldimethylsilyl)pent-4-ynal (**18**) 66
2.3.5. Synthesis of 7-(benzyldimethylsilyl)hept-1-en-6-yn-3-ol (**19**) 67

2.3.6. Synthesis of benzyl(5-((tert-butyldimethylsilyl) oxy)hept-6-en-1-yn-1-yl)dimethylsilane (**20**) 68

2.3.7. Synthesis of 7-(benzyldimethylsilyl)hept-1-en-6-yn-3-ol (**19**) 69

2.3.8. Resolution attempt 2 70

2.3.9. Resolution attempt 3 70

2.3.10. Synthesis of 7-(benzyldimethylsilyl)hept-1-en-6-yn-3-yl acetate (**23**) 71

2.3.11. Resolution to obtain the alcohol (**21**) 72

3. Work done in Spain 73

3.1. Bicyclic ring 6,5 73

3.1.1. Synthesis of 2-(hex-5-yn-1-yloxy) tetrahydro-2H pyran (**2**) 73

3.1.2. Synthesis of Synthesis of benzyldimethyl(6-((tetrahydro-2H-pyran-2-yl) oxy) hex-1-yn-1-yl) silane (**3**) 73

3.1.3. Synthesis of Synthesis of 6-(benzyldimethylsilyl) hex-5-yn-1-ol (**4**) 74

3.1.4. Synthesis of Ethyl (E)-8-(benzyldimethylsilyl)oct-2-en-7-ynoate (**11**) 75

3.1.5. Synthesis of (E)-8-(benzyldimethylsilyl)oct-2-en-7-yn-1-ol (**12**) 76

3.2. Bicyclic ring 5,5, ... 77

3.2.1. Synthesis of Synthesis of 2-(pent-4-yn-1-yloxy) tetrahydro-2H-pyran (**15**) .. 77

3.2.2. Synthesis of benzyldimethyl(5-((tetrahydro-2H-pyran-2-yl) oxy)pent-1-yn-1-yl) silane (**16**) 77

References 79

Appendix 84

Scheme index

Scheme 1 Metabolism of vitamin D in our body. 3
Scheme 2 - Different methods used to obtain the calcitriol or analogs. 12
Scheme 3– Horner-Wittig coupling method to form calcitriol (Hoffmann-La Roche approach). 12
Scheme 4 - Hoffman-La Roche approach to synthetize the A ring. 13
Scheme 5- Lythgoe method to synthetize the calcitriol molecule using Mourino approach. 14
Scheme 6- Okamura's approach of the A ring synthesis. 15
Scheme 7- Trost method to synthetize the calcitriol molecule. 15
Scheme 8- Trost approach for the A ring precursor synthesis. 16
Scheme 9- Suzuki–Miyaura method to synthetize the calcitriol molecule. 16
Scheme 10- Mourino's approach for the A ring precursor synthesis. 16
Scheme 11- Hoffman La-Roche approach for the CD-ring synthesis. 17
Scheme 12- Lythgoe's approach for the CD-ring synthesis. 18
Scheme 13- Trost approach for the CD-ring synthesis 18
Scheme 14- Mourino's approach for the CD-ring. 19
Scheme 15- Retrosynthetic plan for the synthesis of the analogs. 21
Scheme 16- Protection of the alcohols 1 (n=1) and 14 (n=0) to obtain the alcohols 4 (n=0) and 17 (n=1). 30
Scheme 17- Reactional steps for the synthesis of the 6 (n=1) and 19 (n=0) alcohols. 32
Scheme 18- Protection of the alcohols 6 (n=1) and 19 (n=0). In the protection of the alcohol 6 was used DMF and in the reaction of 7 was used as solvent DCM. 33
Scheme 19- Cyclization of the protected alcohol 7 to obtain the ketone 9. 35
Scheme 20- Cyclization of the bicycle[68-69]. 36
Scheme 21- Deprotection reaction to obtain the alcohols 6 and 19. 6 (n=1), 19 (n=0). 37
Scheme 22- Attempt of alcohol 6 resolution using z-Ala-OSu. 38
Scheme 23- Attempt of alcohol 19 resolution. 39
Scheme 24 - Mechanism used to esterify the alcohol in order to obtain diastroreoisomers. 39
Scheme 25- Enzyme binding regions. 40
Scheme 26 - Attempt of alcohol 19 resolution using three different enzymes, Novozym 435, AYS AMANO and PS AMANO. 41
Scheme 27- Esterification reaction of the **19** alcohol. 42

Scheme 28- Resolution of the alcohol 19 using the Sharpless asymmetric epoxidation method..... 44
Scheme 29- Mechanism for the Sharpless asymmetric epoxidation..... 45
Scheme 30- Reactional steps for the synthesis of the alcohols 4 and 17 (4 for n=1 and 17 for n=0). 46
Scheme 31-Oxidation of 4 alcohol and Wittig reaction to form the ester 11..... 46
Scheme 32- Proposed mechanism for the oxidation of alcohol 4 using TEMPO[77-78]. 47
Scheme 33- Proposed mechanism for the Wittig reaction. 48
Scheme 34- Ylide synthesis, new approach. 50
Scheme 35- Phosphorous ylide formation. 50
Scheme 36- Reduction of the ester 11 to the alcohol 12. 51

Figure index

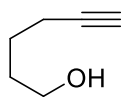
Fig. 1- Chemical structure of Cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2)... 1
Fig 2- Structure and nomenclature of the pro-vitamin D_3 and the vitamin D_3. 2
Fig. 3 -Calcitriol interaction with binding pocket of VDR. Image given by Julian Loureiro. .. 6
Fig. 4-Genomic mechanism of the calcitriol. 7
Fig. 5- Some of the calcitriol analogs with clinical interest[36-37]. 11
Fig. 6- Calcitriol analogs studied. 22
Fig. 7- Representation of the analog **A** interacting with the VDR binding pocket, A, and the overlap of the analog with the calcitriol molecule, B. 23
Fig. 8- Representation of the analog **B** interacting with the VDR binding pocket, A, and the overlap of the analog with the calcitriol molecule, B. 24
Fig. 9- Representation of the analog **C** interacting with the VDR binding pocket, A, and the overlap of the analog with the calcitriol molecule, B. 24
Fig. 10 - Molecule of calcitriol and the wanted analogs. 26
Fig. 11- The corresponding precursors of the previous analogs that we tried to obtain. ... 26
Fig. 12- Synthetic strategy used in Porto to obtain the three wanted precursors. 27
Fig. 13- Synthetic plan used in Santiago de Compostela to obtain the wanted precursors. 29
Fig. 14- Chemical shifts(ppm) for the compound 4. ^{1}H NMR- H and ^{13}C NMR-C. 31
Fig. 15- Chemical shifts(ppm) for the compound 17. ^{1}H NMR- H. 31
Fig. 16- Chemical shifts(ppm) for the compound 6. ^{1}H NMR- H and ^{13}C NMR-C. 32
Fig. 17- Chemical shifts(ppm) for the compound 19. ^{1}H NMR- H and ^{13}C NMR-C. 33
Fig. 18- Chemical shifts(ppm) for the compound 7. ^{1}H NMR- H and ^{13}C NMR-C. 34
Fig. 19- Chemical shifts(ppm) for the compound 20. ^{1}H NMR- H and ^{13}C NMR-C 34
Fig. 20- Chemical shifts(ppm) for the compound 9. ^{1}H NMR- H and ^{13}C NMR-C 37
Fig. 21- Chemical shifts(ppm) for the compound 23. ^{1}H NMR- H and ^{13}C NMR-C 41
Fig. 22- Chromatogram obtained for the racemic acetylated alcohols. 42
Fig. 23- Chromatogram obtained for the alcohol acetylated by the Novozyme 435 43
Fig. 24- Chemical shifts(ppm) for the compound 11. ^{1}H NMR- H and ^{13}C NMR-C 49
Fig. 25- Chemical shifts(ppm) for the compound Y. ^{1}H NMR- H and ^{13}C NMR-C. 50
Fig. 26- Chemical shifts(ppm) for the compound 12. ^{1}H NMR- H and ^{13}C NMR-C. 51

Abbreviations

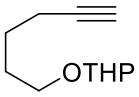
AcOEt	Ethyl acetate
Bn	Benzyl group
DCM	Dichloromethane
DHP	3,4-Dihydro-2H-pyran
DIEA	N, N-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DIPT	Diisopropyl tartrate
DCHT	Dicyclohexyl tartrate
DIBAL-H	Diisobutylaluminium hydride
Et_2O	Dietylether
Et_3N	Triethylamine
Hex	Hexane
MeLi	Methyl-lithium
MeOH	Methanol
n-BuLi	n-Butyllithium
NMR	Nuclear magnetic resonance
NMO	N-Methylmorpholine N-oxide
PTH	Parathyroid hormone
p-TsOH	P-Toluenesulfonic Acid
PPTS	Pyridinium p-toluenesulfonate
PCC	Pyridinium chlorochromate
Ph	Phenyl group
$PhI(OAc)_2$	Diacetoxyiodobenzene

RXR	Retinoid X receptor
R_f	Retention factor
RT	room temperature
TLC	Thin layer chromatography
^{t}Bu	*tert*-butyl
TBSCl	*tert*-Butyldimethylsilyl chloride
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
THF	Tetrahydrofuran
THP	Tetrahydropyran
Ti(^{i}PrO)	Titanium isopropoxide
VDR	Vitamin D receptor
VDR-AP	Vitamin D receptor – Alternative pocket
VDR-GP	Vitamin D receptor - Genomic pocket
VDRE	Vitamin D response elements
VDBP	Vitamin D binding protein
Ƞ	Yield

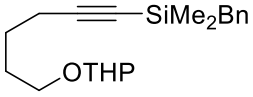
Compounds index



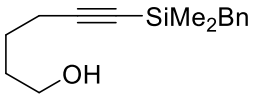
1



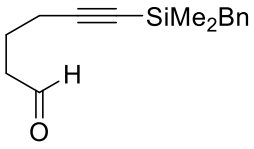
2



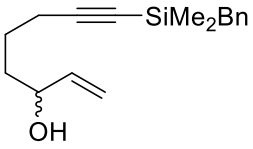
3



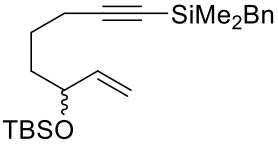
4



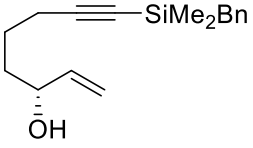
5



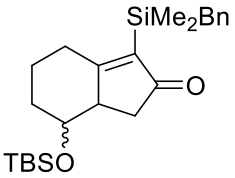
6



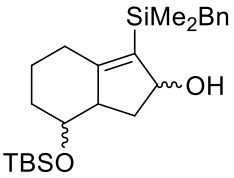
7



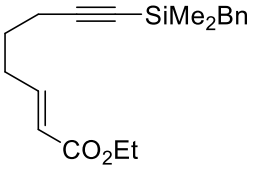
8



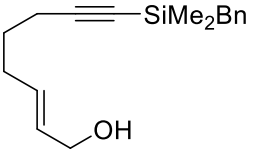
9



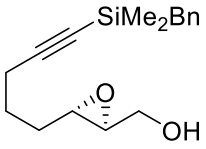
10



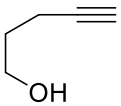
11



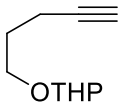
12



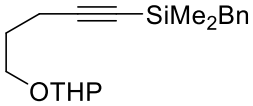
13



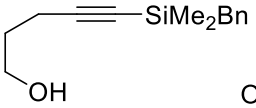
14



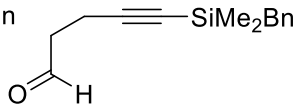
15



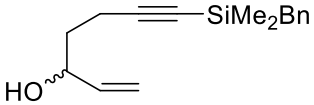
16



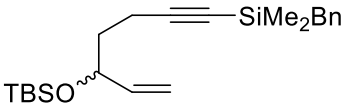
17



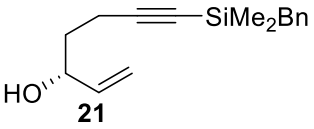
18



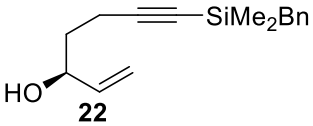
19



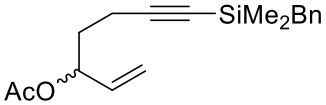
20



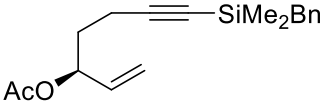
21



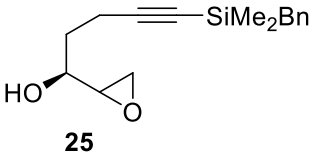
22



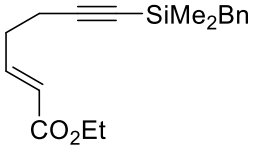
23



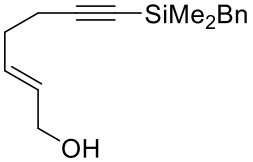
24



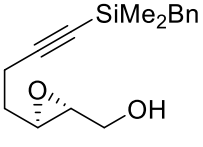
25



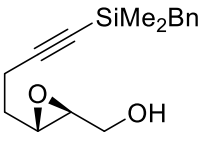
26



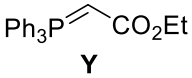
27



28



29



Y

Introduction

1. Vitamin D

1.1. Vitamin D chronology.

The classic bone disease, rickets was first described around 1645 and 1660 by Dr. Daniel Whistler[1]. Prior to 1789, the treatment of rheumatism and rickets, was carried out by the consumption of cod liver oil (known for the great amount of vitamin D)[2]. Only in 1824 D. Schütte proposed cod liver for the treatment of rickets[1].

In 1914 Casimir Funk wrote an article in which he describes that the rickets must occur only when certain substances in the diet are lacking, and these specific substances were present in good breast milk and in cod liver oil. In 1919 Kurt Huldschinsky showed that the ultraviolet rays, from a mercury lamp, could improve rickets in children[3]. Also in 1921 an article was published concluding that exposure to sunlight could treat rickets in children by testing five children with this disease to sunlight [4 5].

Vitamin D has two distinct forms, ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3) (Fig.1). The vitamin D_2 is produced by fungus and its discovery was first published in 1930 by Askew et al. They extracted vitamin D_2 from a mixture of plant sterols and discovered that this compound was active in the healing of the rickets. Also, this group has been successful in isolating vitamin D and in determining the structure of the vitamin D_2 (ergocalciferol)[6].

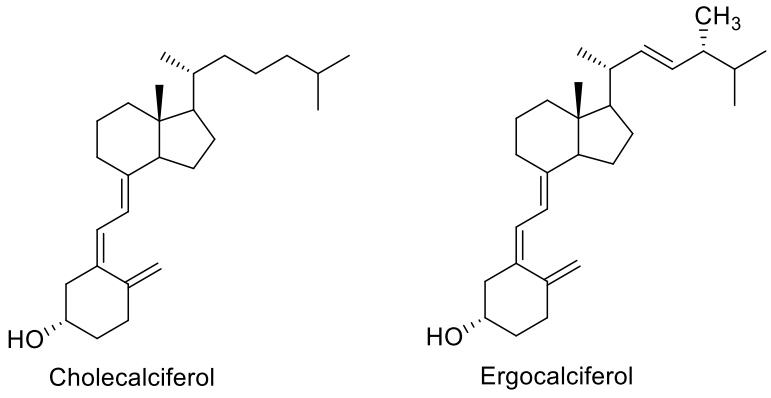
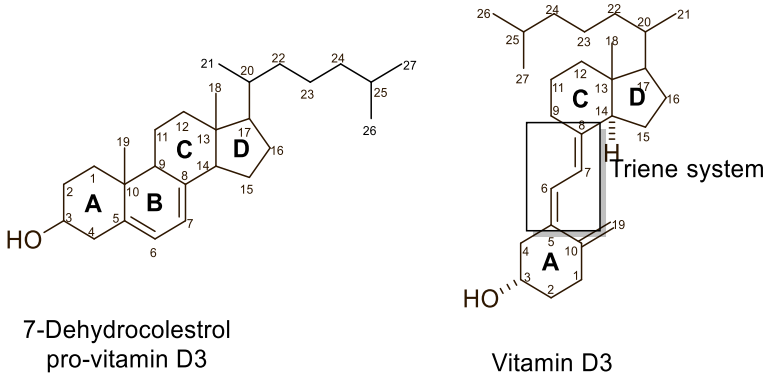


Fig. 1- Chemical structure of Cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2).

Vitamin D_3 (cholecalciferol) is synthesized in our organism and is a secosteroid (9,10 secosteroid)[1]. The structure of this vitamin is influenced by the structure of its precursor, pro-vitamin D_3 (7-Dehydrocolestrol). This precursor has four rings, a, b, c and d (Fig.2). The similarity of the two structures is because provitamin D_3 forms the vitamin D_3 by a photochemical reaction. During the transformation in vitamin D_3, the ring b opens and forms the triene system[7] (Fig.2). In 1936 Windaus and Bock determined the structure of the precursor synthetized in the skin [8-9]. The confirmation that vitamin D_3 is synthetized in the skin was given by Esvelt et al. and Holick et al., by the chemical identification of both vitamin D_3 and provitamin D_3 in the skin[10-11].



7-Dehydrocolestrol
pro-vitamin D3

Vitamin D3

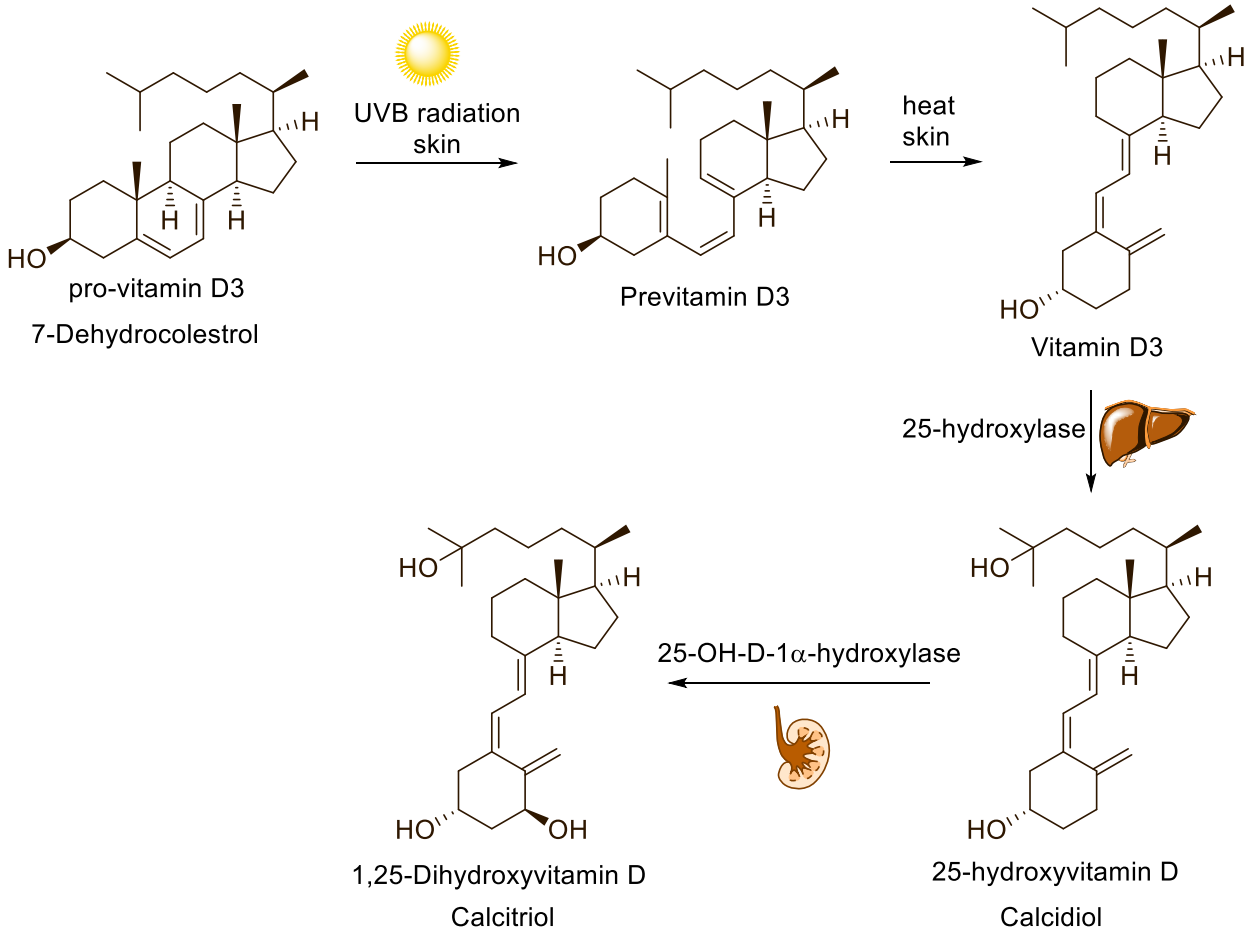
Fig 2- Structure and nomenclature of the pro-vitamin D_3 and the vitamin D_3.

Later in 1938, it was established that the UV light is necessary to synthetize vitamin D in rachitic rats[12]. Since then vitamin D has showed that can be used for the treatment of other diseases, having therefore pharmacologic potential.

1.2. Vitamin D system

Our skin has a lipophilic hormone known as 7-dehydrocholesterol (pro-vitamin D_3), and when exposed to the UV radiation is transformed into previtamin D_3. This molecule is then transformed in Vitamin D_3 (cholecalciferol) with the heat of our skin. Because vitamin D_3 is inert in our body it as to be activated. To became active this vitamin has to be metabolized first in the liver, by the enzyme 25-hydroxylase, and then in the kidney, by the enzyme 25-OH-D-1α-hydroxylase, becoming in this way the active form of vitamin D, the calcitriol[13] (Scheme 1).

[1] Secosteroid is a steroid molecule with a broken ring.



Scheme 1 Metabolism of vitamin D in our body.

In order to be metabolized, the vitamin D_3 must be transported in the blood by the vitamin D binding protein. This binding protein bounds to the vitamin D (both ingested and skin synthetized) in the circulatory system, which will take the vitamin to the liver to be metabolized[14]

In addition to the biosynthesized calcitriol it can, also be, found in food in low amounts. Foods rich in this vitamin are oily fish, like salmon, tuna, sardines, and oils from fish liver like cod, and f sun-exposed mushrooms. Some foods, like margarine, yogurt, infant formula, butter, cheese and breakfast cereals are now fortified with vitamin D. The vitamin D obtained from dietary sources has to be firstly integrated into chylomicra and then transported by the lymphatic system into the venous system[14].

After the formation of calcitriol, it will bind to the VDR receptor, since this molecule has a great affinity to this receptor. This binding will lead to an increase of intestinal absorption of calcium and phosphor [15]. To regulate the amount of calcitriol is necessary the enzyme 24-hydroxylase that degrades both 1,25-$(OH)_2D_3$ and 25-OH-D_3, by the catalysis of

the side chain oxidation. This hydroxylation can start on the C23 or on the C24. In the C23 pathway, exists a hydroxylation at C23 and C26 to get as final product the 1,25-$(OH)_2D_3$-26,23-lactone. In the oxidation by the C24 pathway, it occurs a cleavage of the sidechain, obtaining the calcitroic acid. This last pathway occurs in five steps. After this process these more hidrossoluvel catabolites can be excreted[16-18].

1.3. Function and biological activity of calcitriol

After being ingested or synthetized the calcitriol is transported by the VDBP (Vitamin D Binding Protein) and then binds to the VDR receptor (this binding is responsible for the biological activity). The VDB protein besides the transportation of the vitamin D metabolites is responsible for the protection of calcitriol, increasing its half-life in the serum[19].

1.3.1. VDR Receptor

The VDR receptor belongs to the large nuclear receptor superfamily. This superfamily of nuclear receptors is activated by lipophilic hormones such as steroids, retinoids, thyroid hormones and vitamin D. These hormones are potent regulators of development, cell growth and differentiation, homeostasis and organ physiology. Due to their lipophilicity they can pass through the membrane and bind to the nuclear receptor[20]. This receptor is widely distributed across the body tissues.

The human VDR receptor is a 427 amino acid peptide and has various domains. The first domain is the N terminal (A/B region), then the second domain, the C region a DNA binding domain (DBD), the third domain appears in a flexible hinge region (D region) and the last is the E or E/F domain, where is the ligand binding domain (LBD) [21-22].

1.3.1.1. DNA binding domain

The DBD domain is highly conserved, and consists in two similar modules, containing zinc fingers structures. In each zinc finger the zinc atom is individually coordinated in a tetrahedral way and is stabilized by four highly conserved cysteine residues. These two zinc fingers are apparently structurally similar, but they differ because they have a different chirality of the residues that coordinate the zinc atom in each module. Moreover, these two structures have a different function. The first zinc finger is responsible for the binding of specific DNA to the VDRE (vitamin D response elements) and the second zinc finger serves as the site for heterodimerization of the VDR to the RXR [21, 23].

1.3.1.2. Ligand binding domain

This domain contains the VDR binding pocket, which is responsible for the interaction between the ligand (vitamin D) and the receptor. In the end of the C-terminal there is an activation domain known as AF- 2, which is important for the binding of coactivators and corepressors [23]. When the ligand binds to the LBD, it will induce a change in the conformation of the AF-2 helix, this change will allow the recruitment of coactivators [24].

The overall fold of the VDR LBD is like other nuclear receptors, this LBD has 13 α helices that forms three "sandwich" layers and has three stranded β sheets. This domain goes through major changes when a ligand is bound. When the ligand is bound to the receptor the helix 11 gets the same direction as the helix 10, and the helix 12 (H12) changes their position to seal the binding cavity of the LBD. The residues of the H12, Val-418 and Phe-422, make two van der walls connections with the methyl group of the ligand. The position of this helix is maintained by two polar interactions and several hydrophobic interactions. There are some residues that interact with the ligand and are important to stabilize the position of H12 such as Val-234, Ile-268, His-397 and Tyr-401. The ligand biding pocket is coated by, mostly, hydrophobic residues and the cavity has a volume of 697Å^3, which only 56% is occupied by the ligand. The space of the cavity that isn´t occupied by the ligand is used by two water molecules near the position 2 of the A ring. This additional space can accommodate ligands with the methyl group at the position 2 (shows an increase in affinity of 4-fold greater than the natural ligand). When connected to the VDR receptor the calcitriol molecule is in its active conformation. In this conformation, the A ring of the calcitriol is in a β chair conformation and the 1-OH and the 3-OH are in equatorial and axial orientations. The triene system connecting the A and C rings is adjust in hydrophobic channel sandwiched between, on one side by Ser-275 and Trp-286, and the other side by Leu-233. The hydroxyl group at the position 1 forms two hydrogen bonds with Ser-237 (helix 3) and Arg-274 (helix 5) and the hydroxyl group at the position 3 makes two hydrogen bonds Ser-278 (helix 5) and Tyr-143. The Arg-274 is bounded to water molecules, making a water channel. The 25-hydroxyl group forms two hydrogen bonds with His-305 (helix 6—7) and His-397 (helix 11). The aliphatic chain in the position 17 of the D ring is in an extended conformation that is parallel to the C13-C18 bond and is surrounded only by hydrophobic residues[24-25].

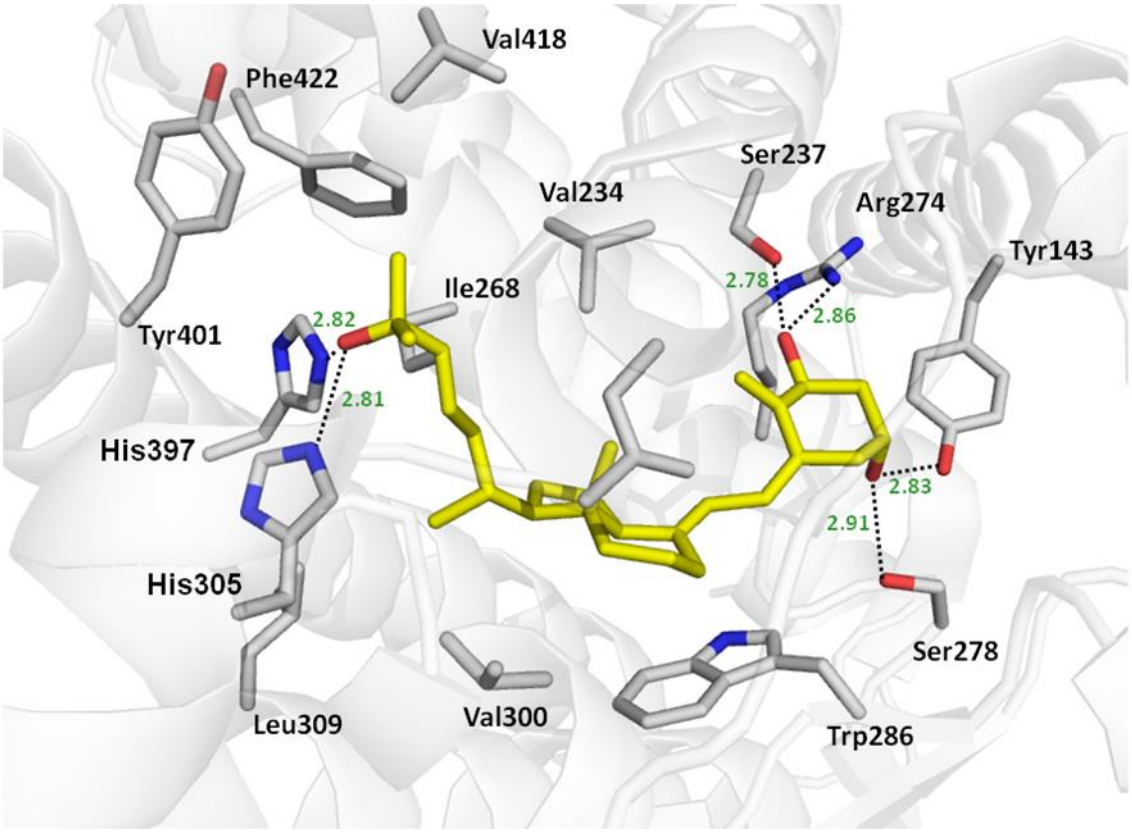


Fig. 3 -Calcitriol interaction with binding pocket of VDR. Image given by Julian Loureiro.

1.3.2. Calcitriol mechanisms of action

As written before, the calcitriol is necessary to regulate various process in the organism. These processes happen in two ways: by the genomic mode, in which the calcitriol interact with the VDR receptor which then interacts with the genome, and by a nongenomic way. These two mechanisms of action have different binding pockets in the receptor. In 2004, it was found a rapid response binding pocket, known as VDR-AP (vitamin D receptor – alternative pocket)[26]. The other binding pocket, the most known, is the genomic response binding pocket (described above), VDR-GP (vitamin D receptor - genomic poket). These two pockets partially overlap.

1.3.2.1. Genomic mechanism

. In the genomic process when the ligand, calcitriol, binds to the receptor the VDR-GP interacts with the RXR to make a heterodimer. This heterodimer then binds to the receptor VDREs (vitamin D response elements) and will lead to an up or down-regulation of these genes. When the ligand binds to the LBD this will induce a change in the conformation of the AF-2 helix which allow the recruitment of a coactivator.

This type of mechanism is responsible for the genomic functions of vitamin D, such as intestinal calcium and phosphate absorption signaling. The genomic mechanism generally takes a few hours to days to manifest and can be blocked by a inhibitor[27-28] (Fig. 4).

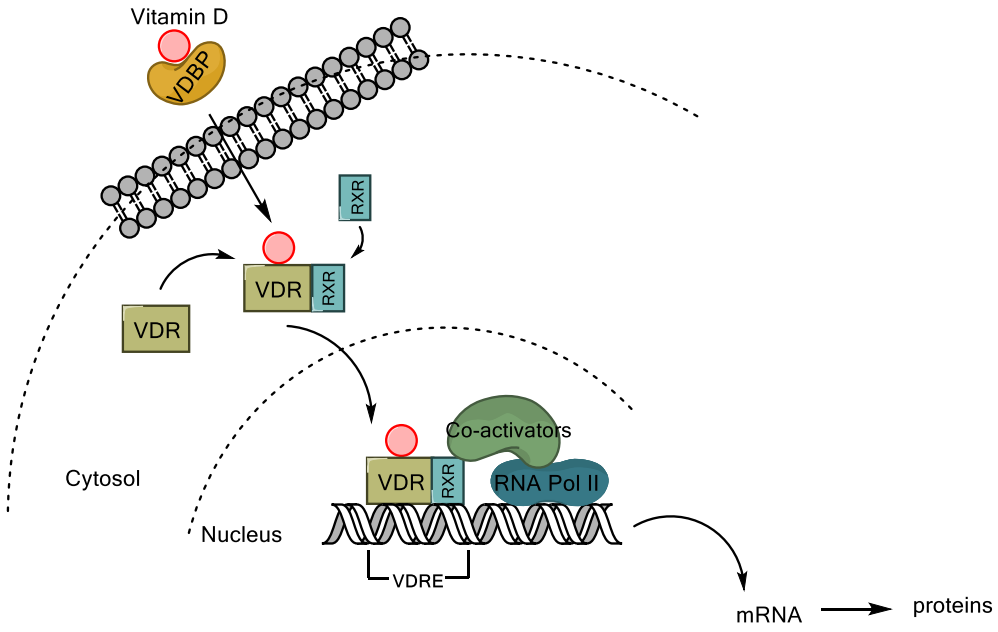


Fig. 4-Genomic mechanism of the calcitriol.

First the vitamin D binding protein transports the calcitriol to the cell. Then inside the cytosol the calcitriol will connect to the VDR and make a heterodimer with the RXR. This heterodimer will bind to VDRE and will connect to a coactivator that binds to an RNA polymerase. Then the target genes transcription will occur, which will lead to specific functions like mineral homeostasis.

1.3.2.2.Nongenomic mechanism

The non-genomic mechanism, known as the rapid-response mechanism, acts much faster than the genomic one. This mechanism can take from various seconds to an hour[27]. In this mechanism the calcitriol binds to a VDR associated with a caveolae of the plasm membrane, instead of binding to a nuclear localized VDR. This biding will result in the activation of one or more second messenger systems involving G-protein-coupled receptors, phosphatidylinositol-3'-kinase (PI3K), protein kinase C (PKC) or modulate the genomic responses (crosstalk). This type of mechanism can lead to an opening of voltage-gated Ca^{2+} and Cl^{-} channels, stimulate exocytosis, rapid intestinal Ca^{2+} absorption, rapid pancreas insulin secretion or activation of MAP kinase, linked to cell differentiation, and

phosphoproteins. This type of responses mediated by the VDR has been only studied in in cell culture[27-28].

1.3.3. Biological activity

The active form of vitamin D, calcitriol, is fundamental to the homeostasis of both calcium and phosphorous.

To occur the mineral homeostasis is required the involvement of the kidney, parathyroid gland, intestine and bone. When exists a decrease in serum calcium the parathyroid glands sense it. In order to restore these levels, this gland will secrete/synthetize the parathyroid hormone (PTH). The PTH hormone sends a signal to the kidney to activate the CYP27B1 (1α-hydroxylase) transcription, which will result in the production of 1,25-$(OH)_2D_3$. Calcitriol will then bind to the VDR receptor and increase the intestinal calcium and phosphate absorption (in the duodenum calcitriol stimulates the calcium channels (TRPV6) which leads to an increase in Ca^{2+} absorption). Both low concentrations of calcium or phosphate in the serum lead to the liberation of PTH hormone[29]. Calcitriol also induces the synthesis of phosphaturic hormone FG23 in bones. This hormone suppresses the renal synthesis of calcitriol by inhibiting the expression of CYP27B1. This hormone also lowers the serum phosphate levels by inhibiting renal phosphate reabsorption[21, 30].

In osteoblasts, the vitamin D is involved in the formation of osteoid matrix and mineralization. The vitamin D is also responsible for the regulation of cellular proliferation and differentiation. These grown-regulation actions of the vitamin D are being studied for the potential roles in the treatment of cancer, regulation of the immune system and autoimmune disease and cardiovascular diseases. These cellular growth-regulating activities are visible in the skin, so this antiproliferative and anti-inflammatory effects of the calcitriol are being studied for the treatment of psoriasis[21].

1.4. Diseases and therapeutical applications

Calcitriol is important for the regulation of various mechanisms in the organism, so, an unbalanced level of calcitriol can lead to diseases. Vitamin D deficiency can lead to some diseases such as rickets, higher risk of fractures and osteoporosis, cardiovascular diseases, hypertension, diabetes, autoimmune diseases, and cancer, such as colon cancer[30].

The excess of vitamin D can be harmful too; one of the major problems associated is hypercalcemia. Hypercalcemia, high levels of calcium in the blood serum, can lead to calcification of the soft tissues, which can cause organ failure and dead [31].

Due to the biological activity of the calcitriol, described above, the calcitriol molecule has been used as a model for the development of new drugs. These new drugs could act in different therapeutical areas such as oncology, bone disease, skin, neuroscience, hematopoietic system, hormone secretion and auto-immune diseases. The major problem associated with the use of calcitriol as a drug is the hypercalcemic effects[30].

2. Calcitriol analogs

To prevent the calcemic effects associated with the calcitriol treatment, some studies had been done with the objective of finding some new analogs. Here the focus will be in analogs with the same basic structure of the calcitriol molecule. Besides this kind of analogs, there exist calcitriol mimetics that don't have any basic structural features of the calcitriol molecule but that show some of the activities of the calcitriol molecule. These calcitriol mimetics had been developed in order to enhance some specific properties such as their anti-proliferative and immunomodulatory capacities[32].

2.1. Structure function relationship of vitamin D

Is necessary to understand the relations between the structure and the corresponding biological activity. This type of information is important to know what we can change in the structure and what is necessary to maintain the desirable effects. In the structure of calcitriol there are three different parts that can be distinguished in the structure: the central rigid CD-bicyclic ring portion, the flexible side chain connected to CD ring at C17 and the flexible seco-B, A ring system.

2.1.1. Conjugated triene (seco-B ring) and A ring

After the side chain modifications, the A ring modification has been the most studied.

In the A ring hydroxyls, the change of the 1α-hydroxyl group configuration for β-hydroxilated 25(OH)D showed that the analog gets essential inactive. Substituting the hydroxyl group by a fluoro group showed an enormous reduction in the biological activity. In other studies that eliminated the portion 10,19, did not showed lack of function, in fact exists a commercial analog known as Zemplar that don't have this portion. This analog has less calcemic effects and retain the capacity of the calcitriol molecule in suppressing PTH in chronic renal failure diseases.

The analog is only able of binding to the VDR receptor, if it shows a trienic system in which the diene bonds align with the tryptophan residue of the VDR[33].

Some known attempts of alter this system by changing the 5,7 portions of the system and replacing the carbons 6 and 7 by nitrogen or doing catalytic reduction, destroyed the activity of the molecule. This shows the importance of the triene system in the activity of the calcitriol molecule and analogs[34].

2.1.2. C/D ring

To study the importance of the CD ring, some studies were made. One of them, in which a calcitriol analog was synthetized without the CD group, the analog did not show any activity. Most of the studies made in which the CD ring was absent, the analogs didn't have activity and the ones that showed some activity had a low activity and it isn't known if these compounds would "survive" once they appear in the circulation. Therefore, the current evidence shows that the removal of the CD ring leads to a complete elimination of the vitamin D activity. However, the methyl group at the position 18 had showed little influence in the biological activity of the vitamin D[34].

To increase the time of the analog in the organism is necessary to make analogs that inhibit the molecule catabolism. Some studies shown that functional changes in the side chain could influence the rate of the side-chain catabolic oxidation. The incorporation of a 16-ene modification was enough to alter the ligand-enzyme interaction and cause significant decrease in the rate of the side chain catabolic oxidation[35].

2.1.3. Side chain

The first generation of calcitriol analogs, were modified in the side chain of the calcitriol due to practical reasons, preserving the A-ring and the 25-OH group. Therefore, there are many analogs with various modifications at the side chain.

When the side chain is reduced to an ethyl group, the receptor binding is reduced, and the in vivo activity disappears. With an increase in the number of carbons at the side chain, the activity of the analog also increases. However, the longer the side chain the greater the calcemic effects. So, for a good vitamin D activity a full side chain and the 25-hydroxyl group is important.

The alteration of the C20 configuration to the unnatural S-configuration showed an increase in the biological activity and an increase in bone resorption activity. Alteration in the C21, C26 and C27 has been studied in vivo. These eliminations change both intestinal calcium absorption and bone calcium mobilization [34].

Strategies such the modification of the C-H group by a C-F group showed a decrease in the catabolism, this is justified since the C-F bond is much stronger and so, less easily

broken. The only disadvantage is that the analogs made with this alteration are similar to the calcitriol molecule, and, therefore, showed the same toxic hypercalcemia. An insertion of a sulfone group in the carbon 26 had shown an increased antiproliferative and transcriptionally activity and low calcemic activity [35].

2.2. Analogs with clinical interest

Some of the calcitriol analogs with clinical interest are shown in the Fig. 5. These analogs are used in treatment of autoimmune diseases such psoriasis and eczema and other most commonly associated like osteoporosis.

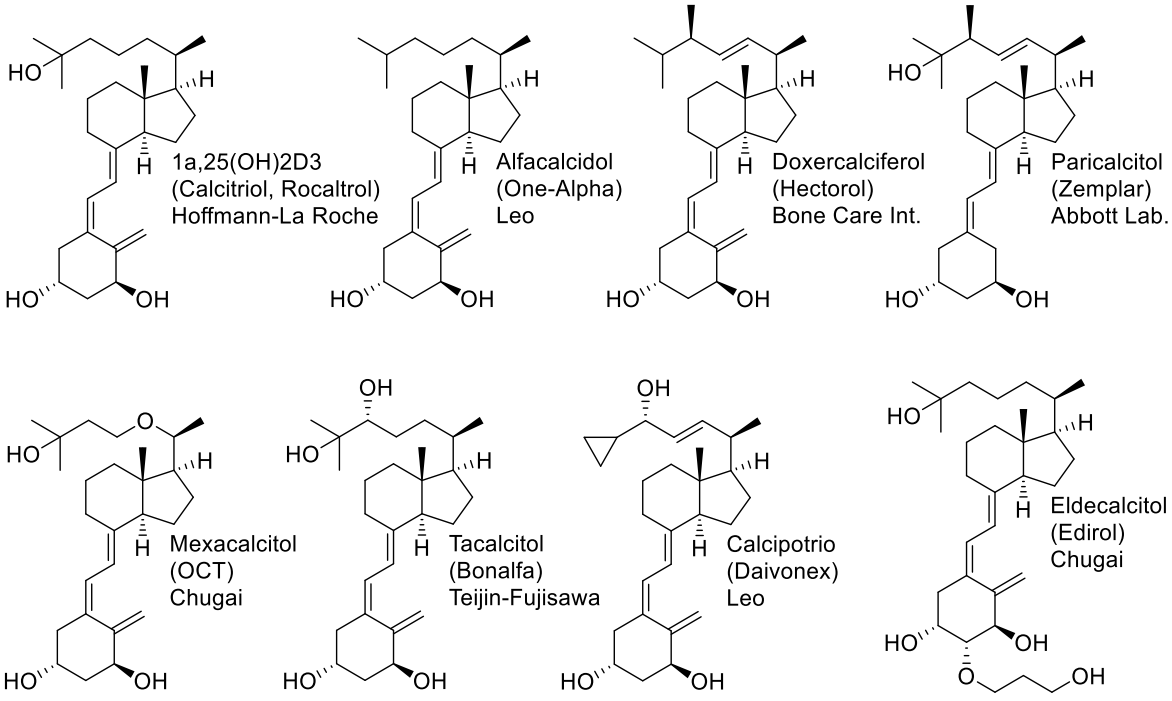


Fig. 5- Some of the calcitriol analogs with clinical interest[36-37].

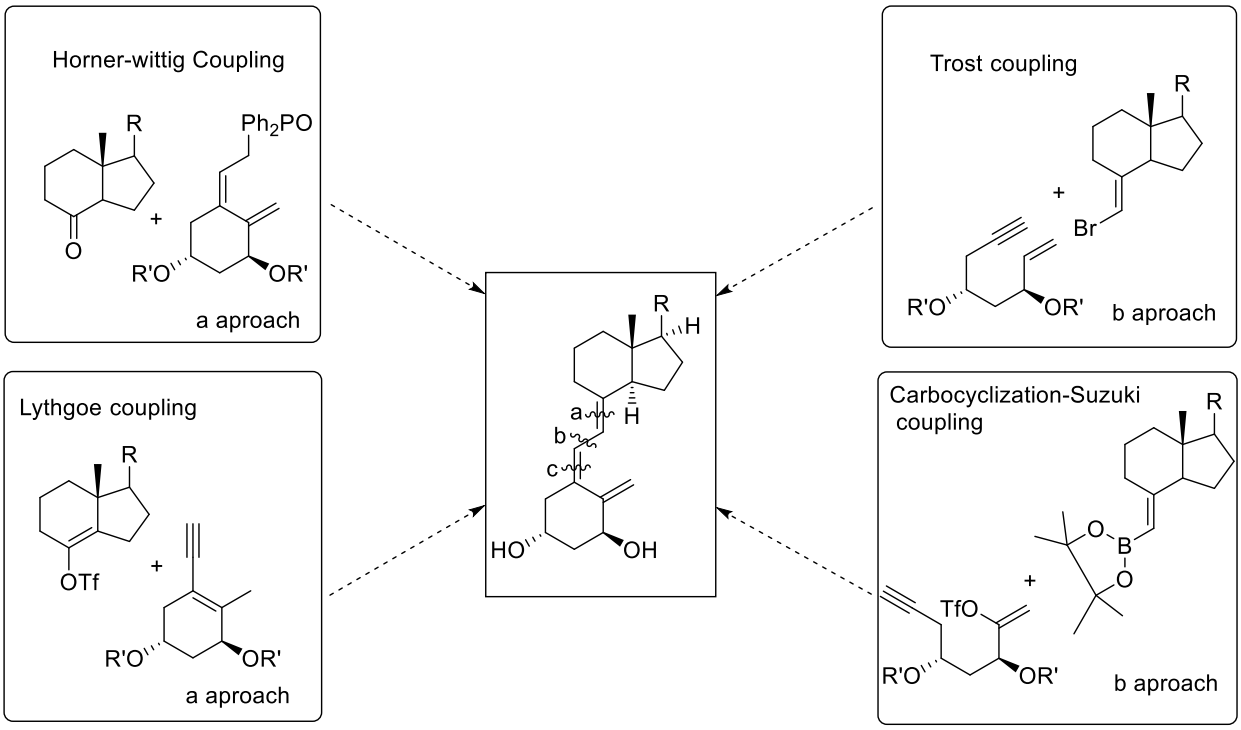
Rocaltrol is the calcitriol molecule sold by the pharmaceutical Hoffman-La Roche. The analogs of this molecule available are the Alfacalcidiol, Doxercalciferol, Paricalcitol, Mexacalcitol, Tacalcitol, Calcipotriol and Eldecalcitol. The Alfacalcidiol, known also as, one alpha, is used for management of hypocalcemia, secondary hyperparathyroidism, and renal osteodystrophy[38]. The Doxercalciferol, known as Hectoral, and the Maxacalcitol, known as 22-oxacalcitriol, are used in the treatment of secondary hyperparathyroidism [39,40]. Paricalcitol, known as Zemplar, is used for the treatment of secondary hyperparathyroidism associated with chronic kidney disease. The Tacalcitol analog, also known as Bonalfa, and the Calcipotriol, known as Daivonex, are used for the treatment of psoriasis. The last one, Eldecalcitol, also known as Edirol, it's sold in Japan for the treatment of osteoporosis[37].

2.3. Synthesis of calcitriol

In this section are explored some of the synthetic methods used to obtain analogs of calcitriol, or to synthetize the calcitriol molecule.

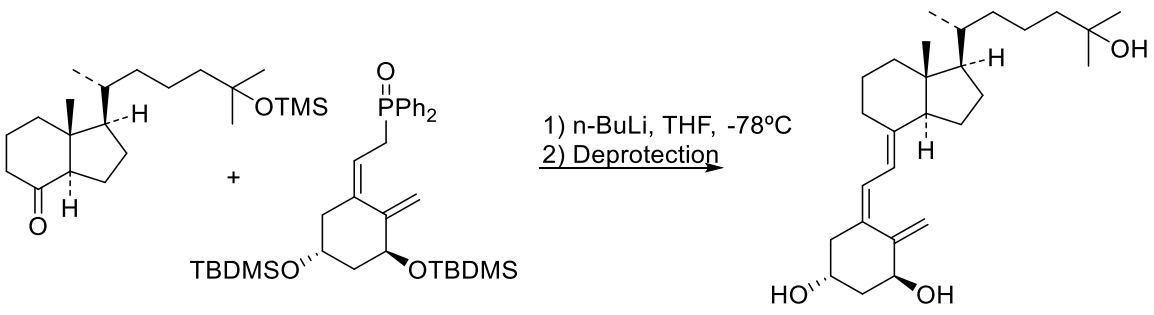
2.3.1. Synthesis of triene unit and A ring

The actual synthetic approaches to obtain some calcitriol analogs are based on convergent methodologies in which a fragment containing an A-ring is attached to a CD fragment[41]. To make the coupling between the A ring and the CD ring exists three approaches, a, b and c. These types of methodologies and approaches are presented in Scheme 2.



Scheme 2 - Different methods used to obtain the calcitriol or analogs.

- Horner Wittig coupling

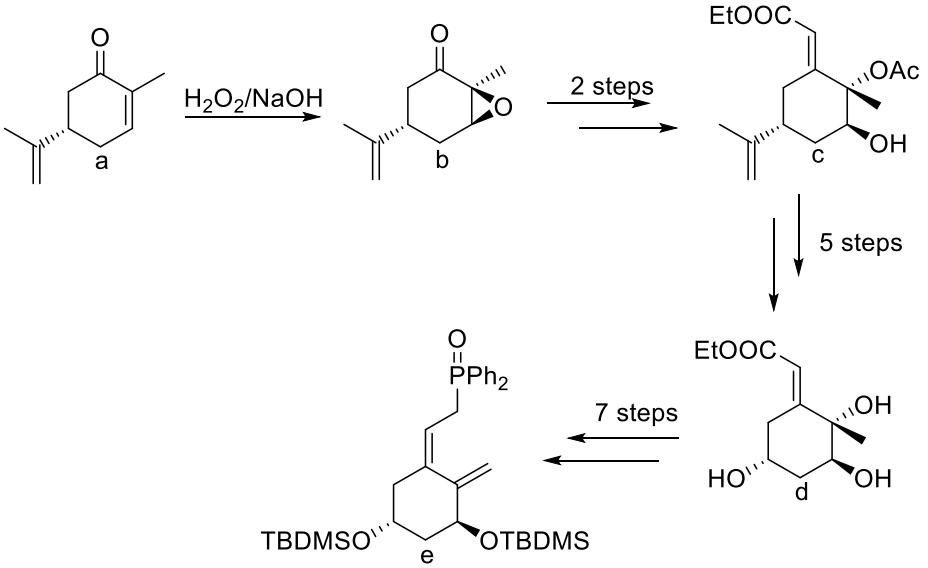


Scheme 3– Horner-Wittig coupling method to form calcitriol (Hoffmann-La Roche approach).

A first approach to this reaction was made by Lythgoe in 1975.This was a Wittig-Horner reaction between the lithium anion of the allylic phosphine oxide (generated by the treatment of the A ring phosphine oxide with n-butyllithium) and the CD-ring Windaus-Grundmann ketone[42]. This approach was further improved by the Hoffmann-La Roche group, to achieve the first total synthesis of calcitriol [43].

The coupling of A ring with the CD ring is obtained in one step at low temperature and takes just 1 hour. Then it is deprotected and these two reactions have an overall yield of 90 %.

Below is represented the A ring synthesis developed by this laboratory. This synthesis is one of the most efficient total synthesis of the 1α-hydroxy A-ring phosphine oxide.

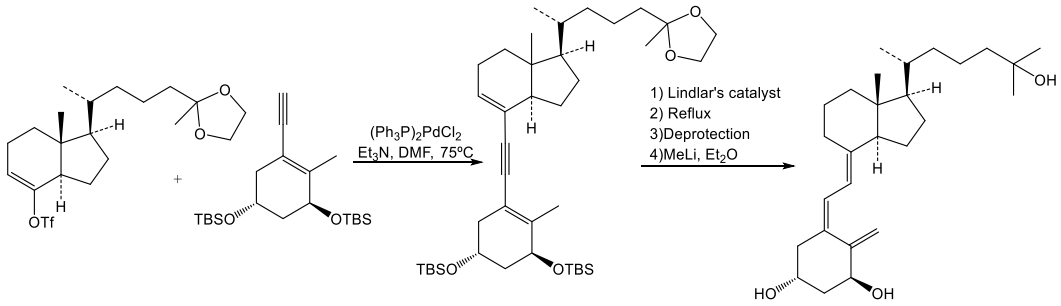


Scheme 4 - Hoffman-La Roche approach to synthetize the A ring.

This 15-steps procedure has an overall yield of 21%. This synthesis starts from the naturally occurring (S)-(+)-carvone, **a**, that goes through an regio and stereoselective epoxidation, **b**, then this epoxide reacts to form an ester epoxide, that is cleaved and esterified, **c**, then an stereoseletive oxidative cleavage of the isopropenyl side chain is done, **d**, and the hydroxyl groups protected. The E-ester is isomerized to the Z-ester and with more 4 steps the phosphine oxide is obtained[42, 44].

- Lythgoe coupling

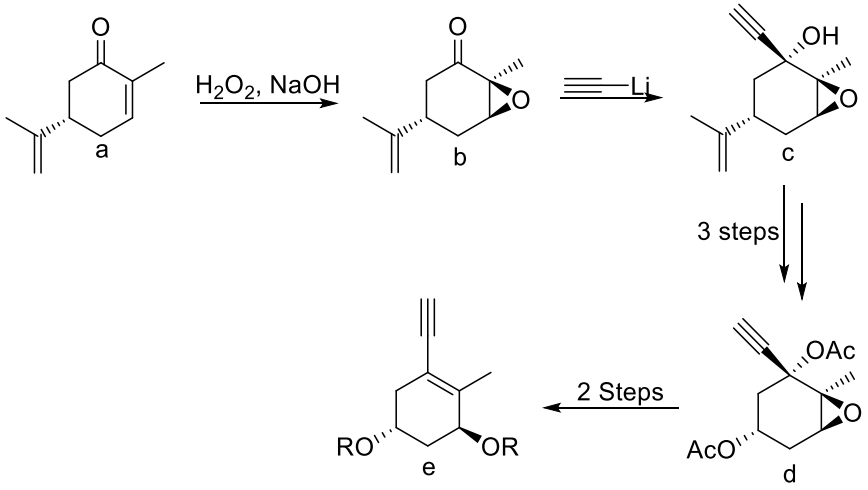
This reaction was first described by Lythgoe in 1971[45] and consists in the coupling of an enantiomerically pure A-ring enyne with a CD-ring 9-chloro ketone through acid workup. The precalciferol is then obtained by semihydrogenation of the acetylene bond, using a Lindlar catalyst. This approach gave an overall yield of 22 %. Later, in 1980, Okamura made another approach.



Scheme 5- Lythgoe method to synthetize the calcitriol molecule using Mourino approach.

Only in 1986, Mourino and his group made another improvement to the Lythgoe's approach by using instead of the CD-ring ketone a CD-ring vinyl triflate[46]. The Mourino approach is described above, Scheme 5, and it is the best version of enyne coupling. In this approach the synthesis of the CD-ring 9- chloro ketone is eliminated (this synthesis has a low yield and requires 7 steps), and the formation of undesired isomers in the coupling is not significant[42].

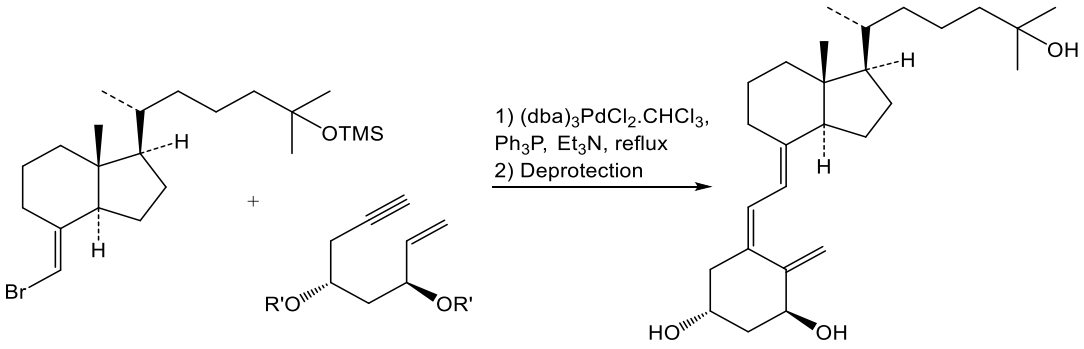
The synthesis of the A ring is showed below, and it is used an Okamura approach- This procedure has an overall yield of 37%[47].



Scheme 6- Okamura's approach of the A ring synthesis.

In this synthesis first occurs the epoxidation of S-(+)-carvone to give cis-carvone epoxide, **b**. Then lithium acetylide is added, to afford the propargyl alcohol, **c**. The hydroxyl group is acetylated and occurs an ozonolysis of the isopropenyl side chain followed by a direct acetylation and an in situ Criegee rearrengement to afford the key diacetate, **d**. The SmI2-promoted reductive elimination of **d** and concomitant ring opening of the epoxide to afford **e**[42, 47].

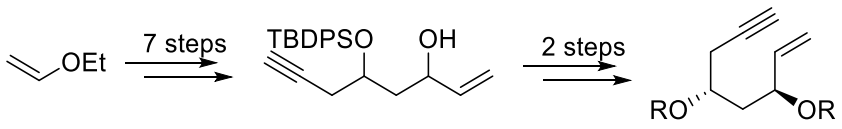
- Trost coupling



Scheme 7- Trost method to synthetize the calcitriol molecule.

This new method for the calcitriol synthesis was described in 1992 by Trost[48]. This approach is a one pot reaction and uses the Pd-catalyzed alkylative cyclization that closes the A ring and forms the bond that connects the CD ring unit with the acyclic unit, this acyclic unit, is the precursor for the A ring. This reaction has an overall yield of 52%[42].

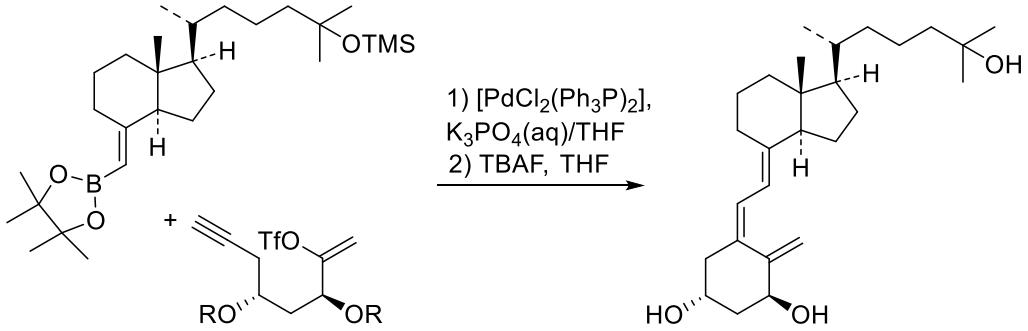
In the Scheme 8, it is represented the synthesis of the acyclic unit, precursor of the A ring.



Scheme 8- Trost approach for the A ring precursor synthesis.

This synthesis has a yield of 20 %. In this reaction, the enantiomerically pure enyne was obtained by using a Sharpless epoxidation with a 46 % yield[42].

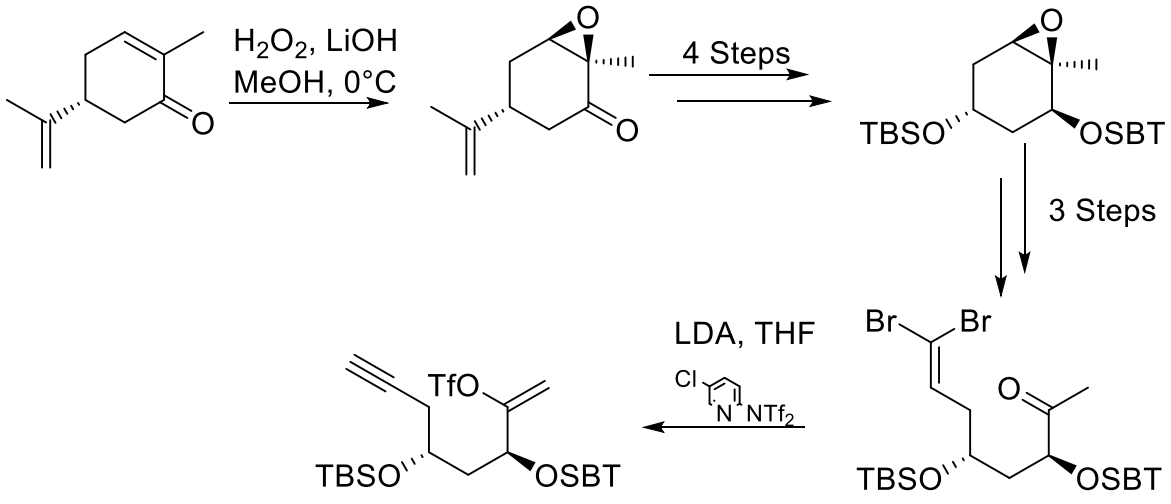
- Carbocyclization-Suzuki coupling



Scheme 9- Suzuki–Miyaura method to synthetize the calcitriol molecule.

This reaction was developed at Mourino's laboratory and has a yield of 72%. This coupling has only two steps. This aproach is characterized by a highly stereoselective intramolecular cyclization of an enol triflate (A ring precursor), followed by a in situ Suzuki–Miyaura coupling of the resulting palladium intermediate with an alkenyl boronic ester (CD ring and side-chain)[49].

The synthesis of the precursor of the A ring is below, Scheme 10. This method has a total yield of 29%.



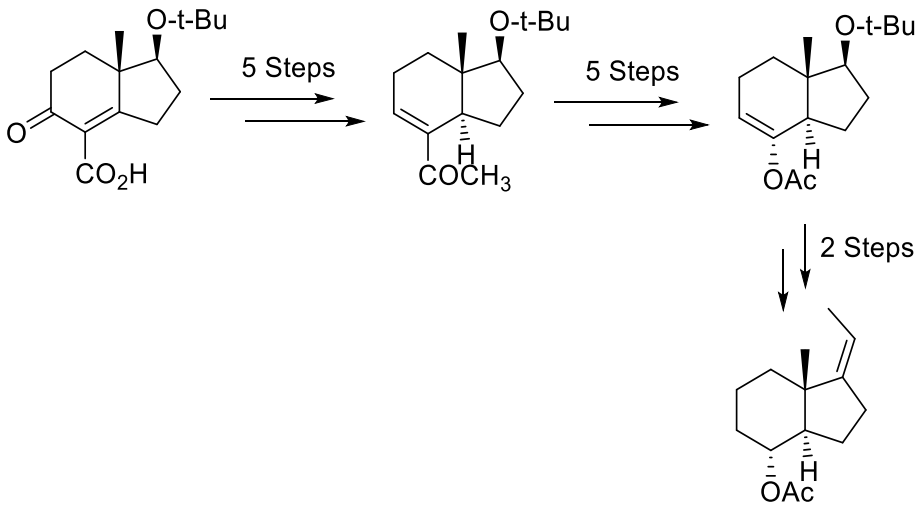
Scheme 10- Mourino's approach for the A ring precursor synthesis.

The synthesis of the A ring precursor starts with the l-carvone, this compound is epoxidized and forms a protected epoxide. Then the oxidative cleavage of the epoxide is performed with periodic acid in diethyl ether. The final step is the conversion of the aldehyde into the desired vinylic triflate[50].

2.3.2. Enantioselective syntheses of the CD-ring

The synthesis of the CD rings of the calcitriol molecule (trans-hydrindane system) is difficult. The major problem is the synthesis of the trans-stereocenter at the ring junction[51]. Some strategies were developed to synthesize this moiety.

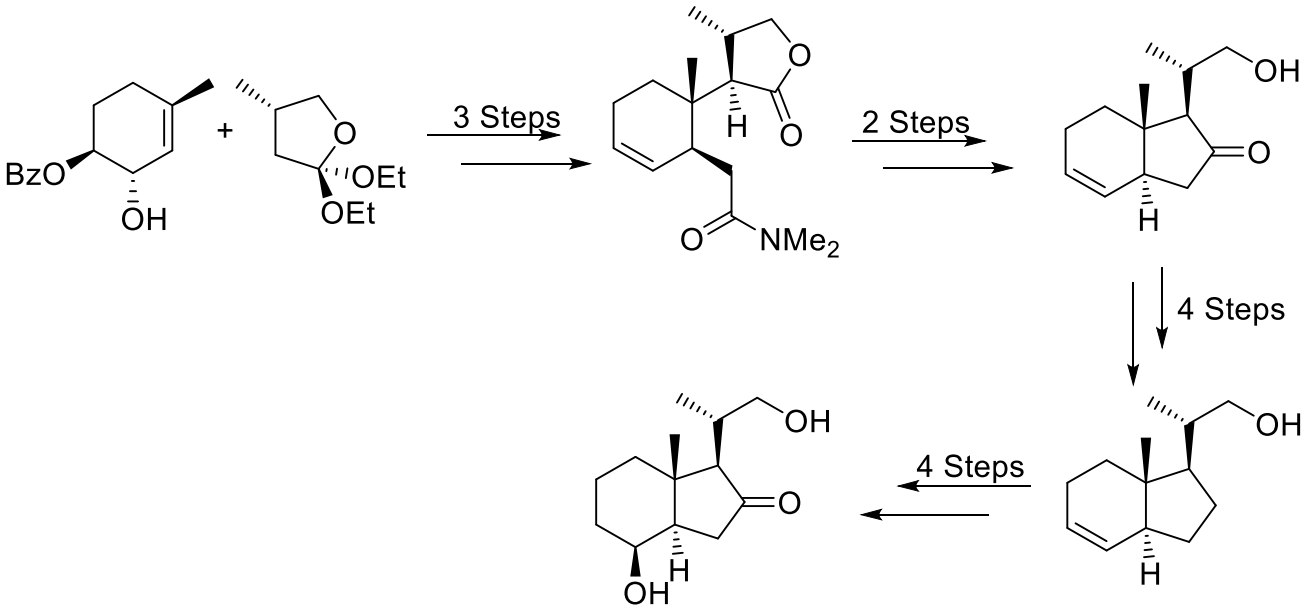
- Hoffman-La Roche approach



Scheme 11- Hoffman La-Roche approach for the CD-ring synthesis.

This approach was done by the Hoffman- La Roche laboratory[52]. The starting material on this approach is a keto acid that was originally synthesized from an enedione[53]. In this process the starting material is hydrogenated stereoselectivity to form the corresponding trans-hydrindane derivative.

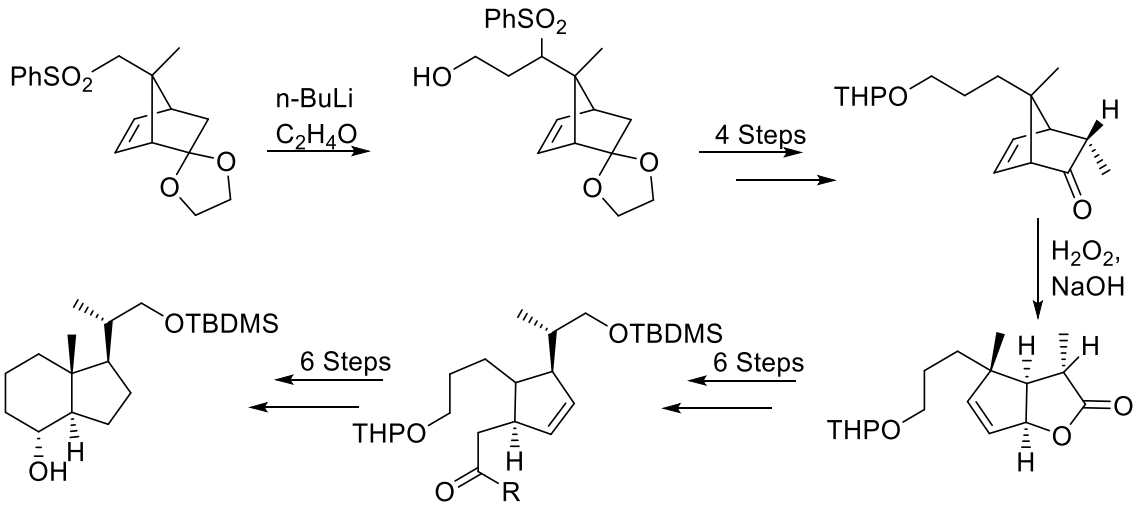
- Lythgoe approach



Scheme 12- Lythgoe's approach for the CD-ring synthesis.

Lythgoe was the first to develop a total synthesis of the Inhoffen- Lythoe diol[54]. In this approach the two starting materials, the orthoester and the allylic alcohol reacts, through a Claisen rearrangement, to give the γ-lactone. This compound is then hydrolyzed and methylated to the corresponding ester. Then a cyclization occurs after a reduction to give the desired Inhoffen-Lythgoe diol[44].

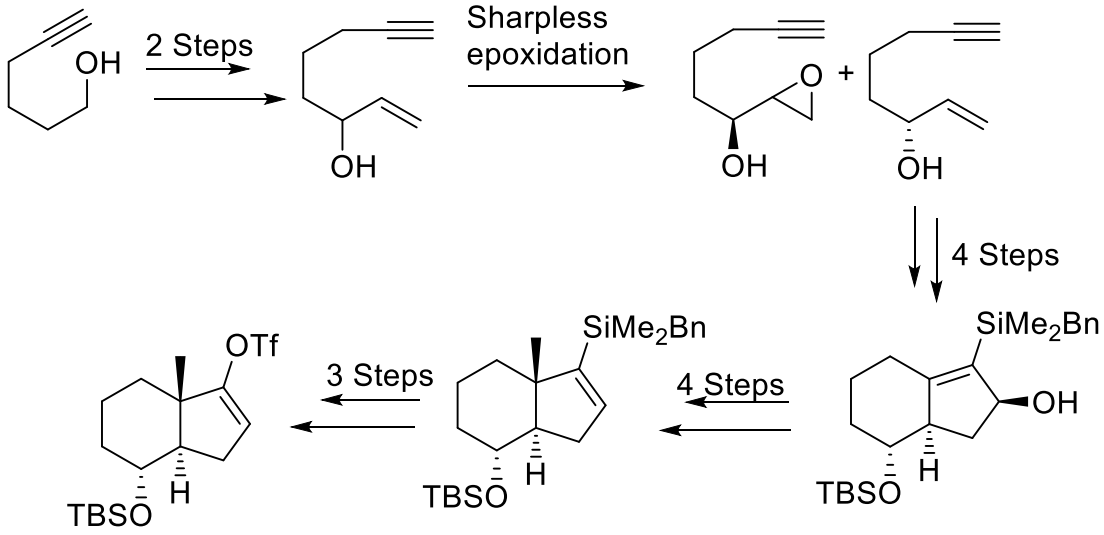
- Trost approach



Scheme 13- Trost approach for the CD-ring synthesis

This synthetic method was developed by Trost and has a yield of 8%. In this approach the synthesis starts with a sulfone, that undergoes two alkylation's, posterior desulfonylation and protection of the alcohol. This compound is then oxidized, using the Baeyer-Villiger oxidation, giving the lactone. The lactone is reduced to a diol and the primary alcohol is protected. Then, by a Claisen rearrangement the trans-ring junction is formed. Later by the removal of the THP protection group the trans-hidrindane system is formed. This last product undergone some other steps like, hydrogenation and oxidation to obtain the expected alcohol.

- Mourino's newest approach



Scheme 14- Mourino's approach for the CD-ring.

This approach was developed by Mourino's laboratory and has a total yield of 8%. The most important steps in this reaction are: The Sharpless epoxidation reaction, needed to obtain the desired alcohol, the Pauson-Khand reaction, super important for the cyclization of the compound to obtain the desired bicyclic ring, and the cuprates reaction, important to insert the methyl group in CD junction[55].

3. Bioavailability

In drug development is important to ensure that the "new drug" is absorbed to ensure its therapeutical effect. The definition of bioavailability is the fraction of drug that gets unchanged to the circulatory system. The bioavailability of the drug is associated with the administration route, is assumed that the intravenous route has a bioavailability of 100 %.

The administration routes used are intravenous, intramuscular, subcutaneous, oral, rectal, transdermal and inhalation.

The new drug must pass through 4 important steps, absorption, distribution, metabolism and excretion (ADME). The performance in this steps will indicate the reliability of the new drug.[56]

To improve the probability of a drug being accepted as a new drug, the docking studies, that evaluate the interactions of the future drug with the corresponding receptor, and other studies such the evaluation of the properties of the molecules have been done with the objective to improve this first process of "finding" the most adequate molecule

In 1997 were found by Lipinsky 5 rules that evaluate the druglikeness of a drug. These rules predict that a drug is more likely to show low absorption when exists more than 5 hydrogen bond donors and more than 10 hydrogen bond accepters, has a molecular weight higher than 500 Da and the LogP is bigger than 5. These rules evaluate the capacity of a molecule to pass through the membrane lipid bylayer[57].

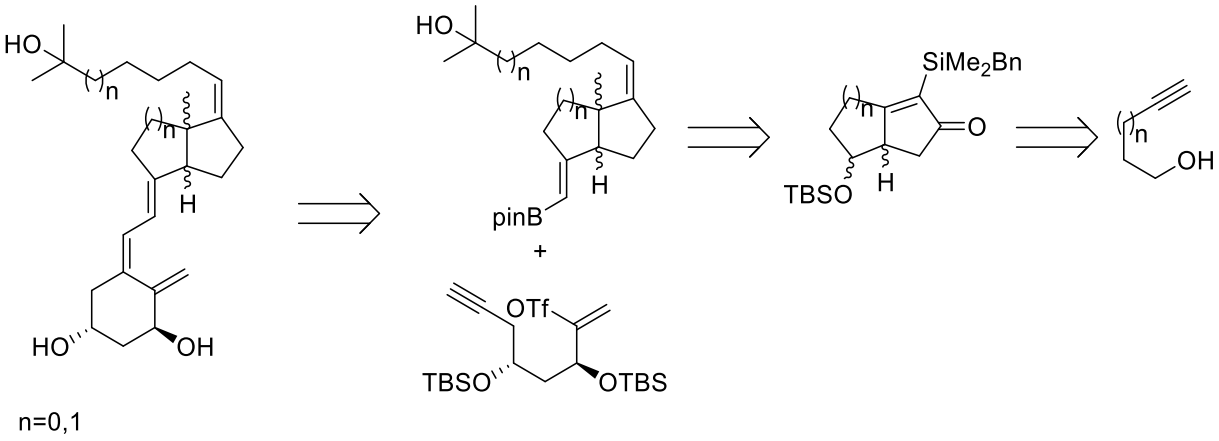
The use of this rules makes the found for new possible drugs easier, but at the same time can eliminate some compounds that would be important. These rules don't work for all the drugs known. Some drugs have molecular weights higher than 500 Da and can be orally absorbed

New studies were done that showed exceptions to this rule, and these rules have been enlarging over the years.[58].

Objectives

In this work, it is proposed the synthesis of precursors of the vitamin D analogs that, according to the previous docking studies may have a good interaction with the vitamin D receptor. The main objective in this work is the synthesis and resolution of synthetic precursors of the enantiomerically pure CD bicyclic rings, using different approaches.

The retrosynthetic plan for this work is represented in the Scheme 15.



Scheme 15- Retrosynthetic plan for the synthesis of the analogs.

This thesis was done in collaboration with the “Faculdad de Quimica da Universidade de Santiago de Compostela” thanks to the Erasmus + program.

Results discussion

1. Docking

In order to know the most adequate analogs to be synthetized was important to do some docking studies. These docking studies were done with the help of my investigation group and with the help of the computational chemistry/biochemistry investigation group of the “Faculdade de Ciências da Universidade do Porto”.

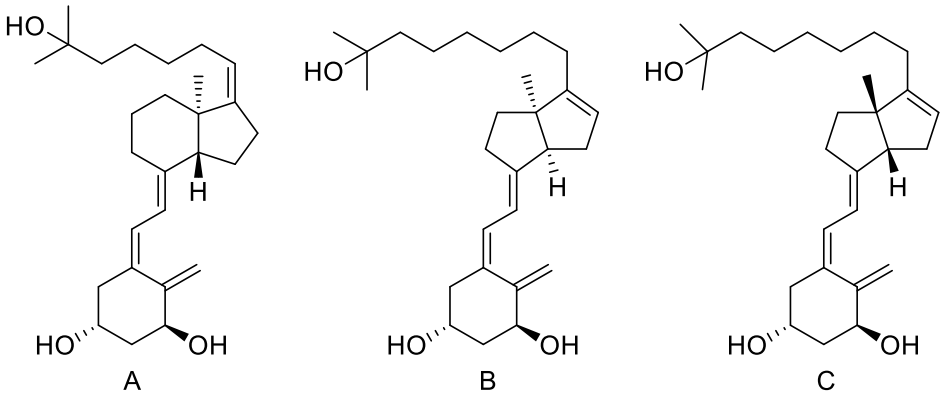


Fig. 6- Calcitriol analogs studied.

The molecules chosen for these docking studies are represented in the Fig. 6. The analog A differ from the original calcitriol molecule in the junction of the hydrindane system, where it has the opposite configuration. Then, in this analog lack’s a methyl group in the lateral chain and appears a new double bond. The other difference is that the lateral chain of the analog **A** has 6 carbons.

The other two molecules, **B** and **C**, differ from the calcitriol, mainly in the bicycle CD. The calcitriol molecule shows a 6,5 bicyclic ring but in these two analogs the bicycle appears with a 5,5 bicyclic ring and a double bond. The lateral chain has 7 carbons. The great difference between the analogs **B** and **C** is the configuration of the CD ring.

For this docking study we used the crystal structure of the nuclear receptor for vitamin D (without the flexible insertion domain) bound to its natural ligand, calcitriol, made by Dr. Moras and his team in 2000[24].

The molecules were drawn using ChemDraw v16.0 and undergone an MM2 minimization in the ChemDraw 3D v16.0. This last step was done to obtain the initial conformations of the molecules. This process was repeated to the calcitriol molecule.

To evaluate the interactions between the analogs with the binding pocket of the vitamin D receptor the program GOLD was used [59]. This software attributes a punctuation corresponding to the interactions of the small molecule with the aminoacids of the binding pocket of the receptor. The obtained results are compared with the punctuations obtained for the original ligand, the calcitriol. The punctuation for the calcitriol is considered 100%.

1.1. Docking results for the calcitriol analog A

The docking punctuation for this analog was 95%. Which means that the molecule doesn't fit so good as the original, but still fits good in the binding pocket of the vitamin D receptor. In the Fig. 7 is represented the interactions of the analog 1 with the aminoacids of the VDR binding pockets, and the overlap with the calcitriol molecule.

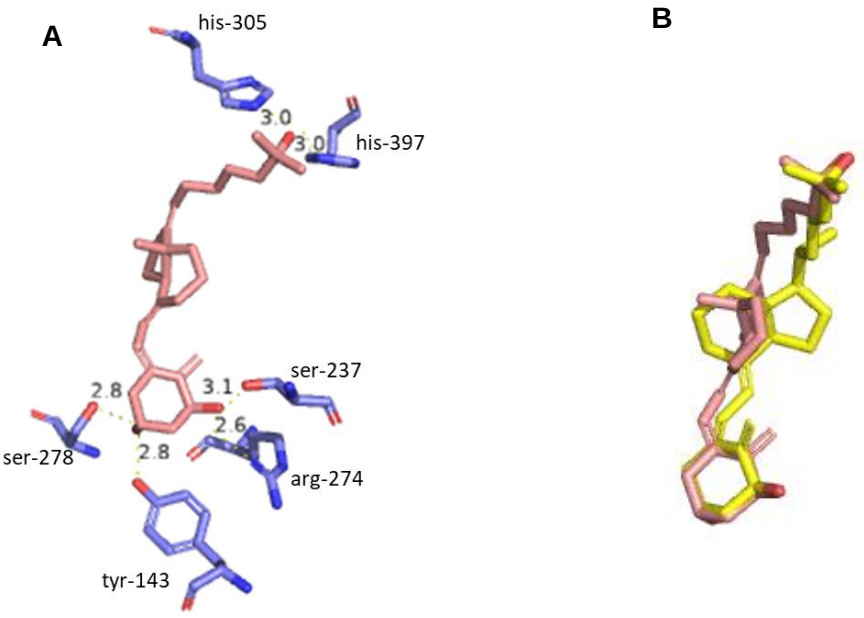


Fig. 7- Representation of the analog **A** interacting with the VDR binding pocket, A, and the overlap of the analog with the calcitriol molecule, B.

1.2. Docking results for the calcitriol analog B

The docking punctuation for this analog was 103%. Which means that the molecule fits even better than the original ligand in the binding pocket of the vitamin D receptor. In the Fig. 8 it is represented the interactions of the analog with the aminoacids of the VDR binding pockets, and the overlap of the calcitriol molecule with the analog.

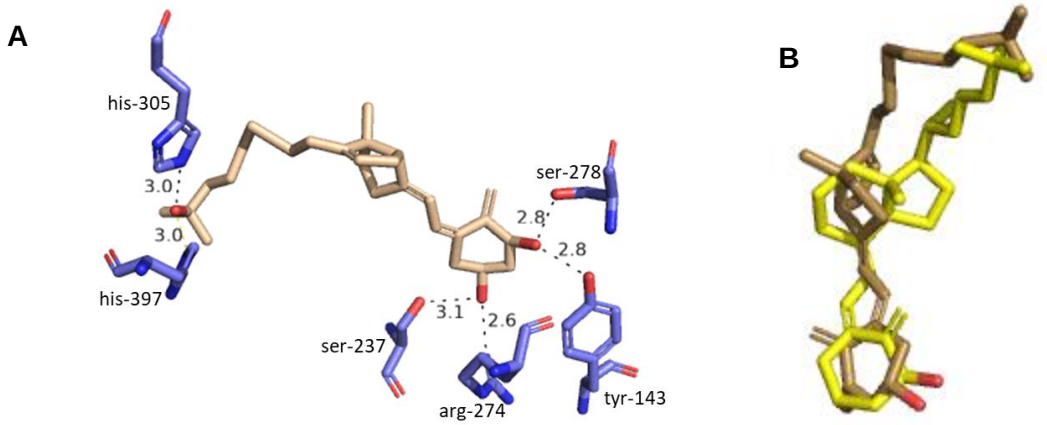


Fig. 8- Representation of the analog **B** interacting with the VDR binding pocket, A, and the overlap of the analog with the calcitriol molecule, B.

1.3. Docking results for the calcitriol analog C

The analog **C** had a docking punctuation of 104%. This better punctuation is probably due to the configuration of the analog, since its configuration is much more similar to the calcitriol molecule. In Fig. 9 is represented the analog principal interactions with the VDR binding pocket and the overlap of the analog with the calcitriol molecule.

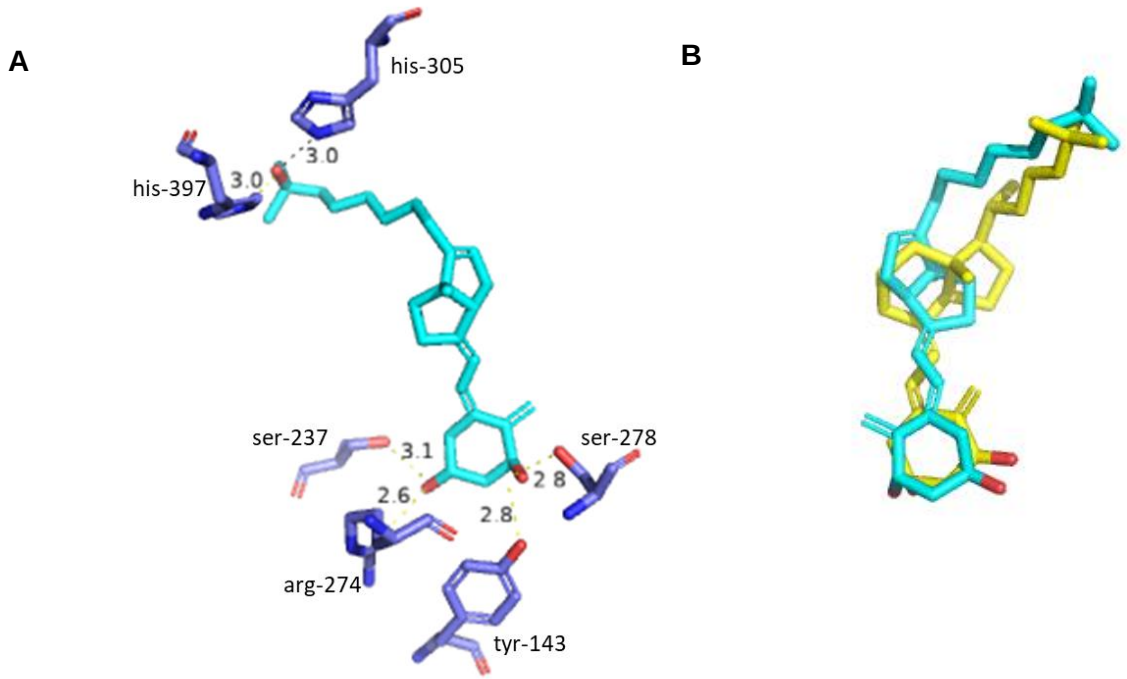


Fig. 9- Representation of the analog **C** interacting with the VDR binding pocket, A, and the overlap of the analog with the calcitriol molecule, B.

1.4. Analogs results comparison

The major difference between these tree analogs is that the analogs **B** and **C** make different interactions with the aminoacids when compared with the analog **A** and the original ligand. The hydroxyl group at C25 in the analog **A** and calcitriol, interacts with the aminoacids Serine 237 and Arginine 237, and the other hydroxyl group at C3 position interacts with the serine 278 and Tyrosne 143. In the analogs **B** and **C** happen the opposite, the hydroxyl group at C25 interacts Serine 237 and Tyrosine 143, the other hydroxyl group interacts with the other two aminoacids. The analogs **B** and **C** differ from each other only in the configuration but show the same interactions with the aminoacids. However, these two analogs show different docking punctuation, being the punctuation better for the analog **C**.

The analog **A**, is much more similar to the calcitriol molecule, but shows the lower docking punctuation, this can be due to the double bound in the lateral chain that require a larger lateral chain, when compared with the original ligand, in order to make good interactions with the both histidine's. The other major difference between both molecules is the configuration of the bicycle CD, as we can observe in figure 7 that gets a total different torsion when compared with the calcitriol molecule.

2. Strategy and synthetic plan

The work presented in this thesis aims to synthetize 3 different calcitriol analogs Fig. 10. Therefore, in this project we have tried to synthetize the 3 different precursors presented in the Fig. 11, by using Mourino's approach.

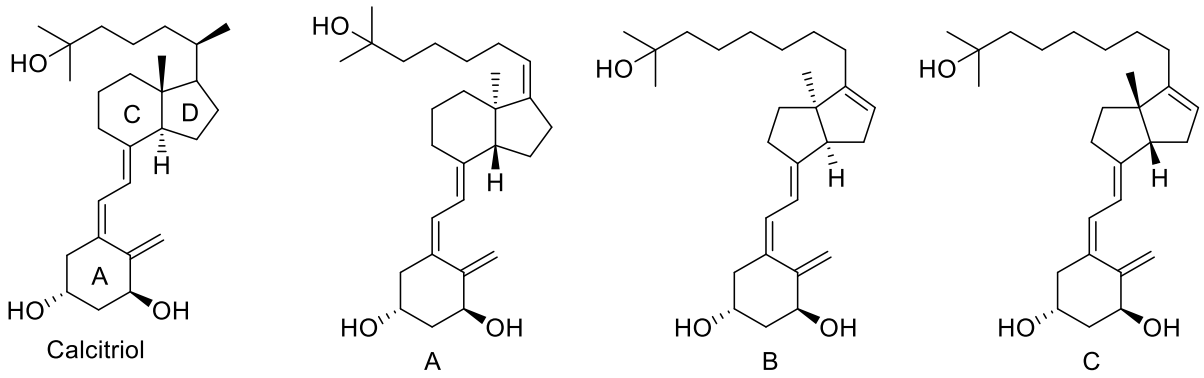


Fig. 10 - Molecule of calcitriol and the wanted analogs.

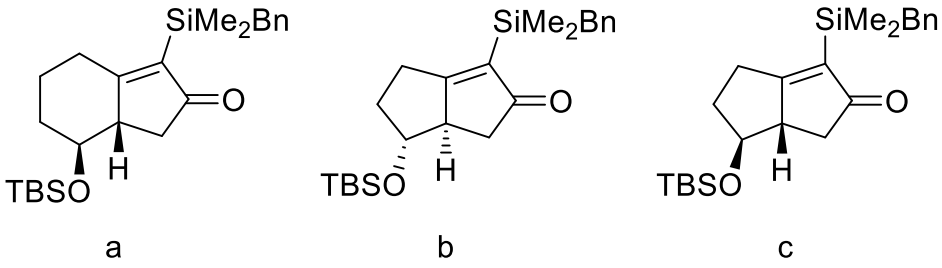


Fig. 11- The corresponding precursors of the previous analogs that we tried to obtain.

For the synthesis of the analogs of these bicyclic rings we started with the corresponding alcohols. For the precursor **a** we started with the 5-hexyn-1-ol to obtain the 6,5 bicyclic ring. For the precursors **b** and **c** started with the 4-pentyn-1-ol to obtain the 5,5 bicyclic ring.

In this project we tried different approaches to obtain the precursors. The first strategy to be explained in this section is the strategy done in Porto. The main route used in this strategy had already been done in Santiago de Compostela[60]. In Porto we tried different strategies to be able of resolving the wanted molecules. This strategy is shown in Fig. 12.

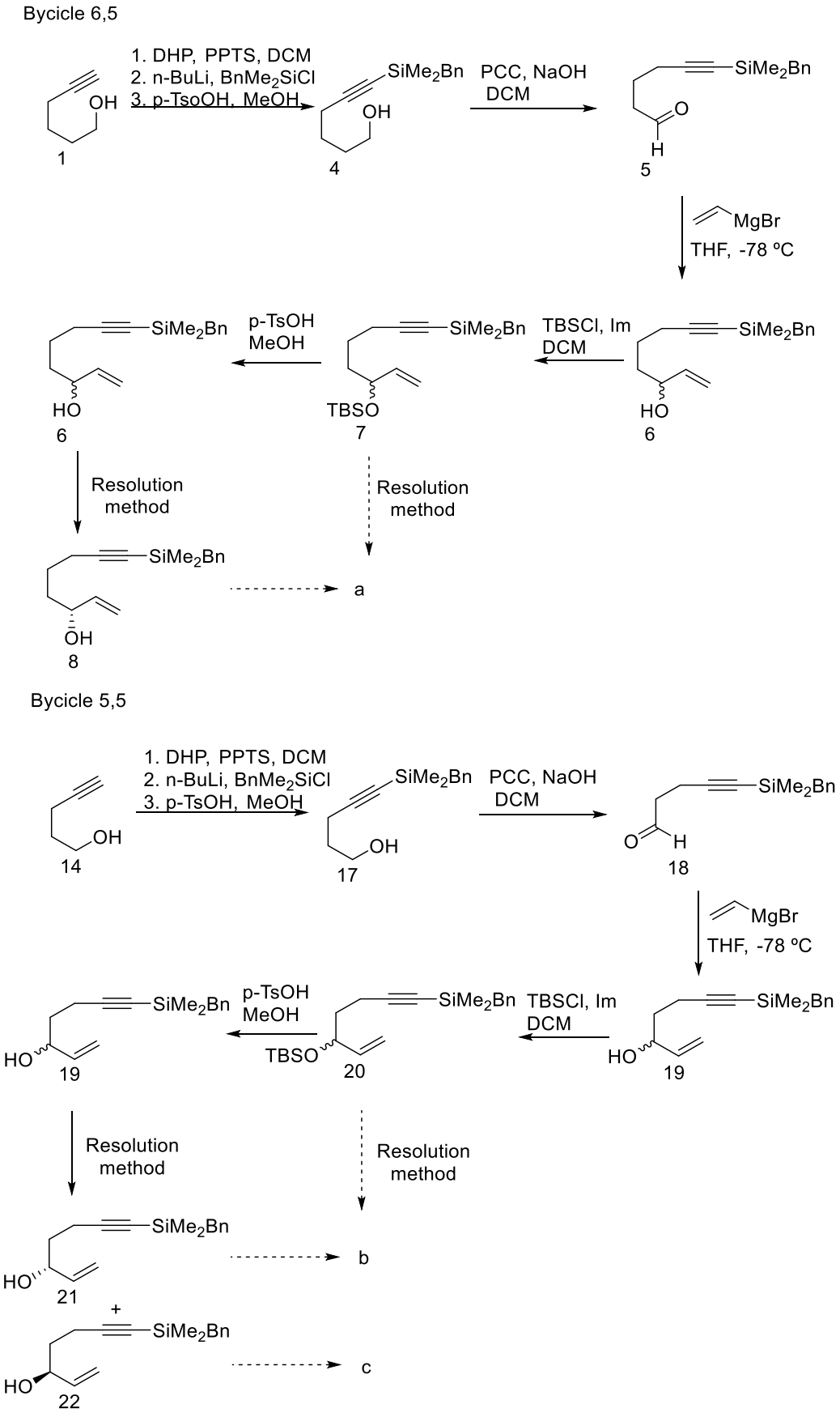


Fig. 12- Synthetic strategy used in Porto to obtain the three wanted precursors.

In this method the synthesis starts with the alcohols 5-Hexyn-1-ol and the 4-Pentyn-1-ol, **1** and **14.** These alcohols were first protected to improve the final yield of the reaction, **4** and **17**. After the protection, the compounds **4** and **17** were oxidized to the aldehydes **5** and **18**. These aldehydes, **5** and **18**, were alkylated using a Grignard reagent and the alcohols **6** and **19** were formed. These alcohols were also protected to undergo the first attempt of resolution, the isocyanate resolution method. This resolution method failed, so, we had to deprotect the alcohols **7** and **20**, to obtain again the **6** and **19** alcohols. After having the alcohol deprotected we tried different resolution methods, such as enzymes, esterification with chiral molecules, and a Sharpless epoxidation.

The next strategy explained in this section is the strategy done in Santiago de Compostela This strategy has been done before, just for the analog **1**, and showed to be very promising[61]. The synthetic plan used is in the Fig.13.

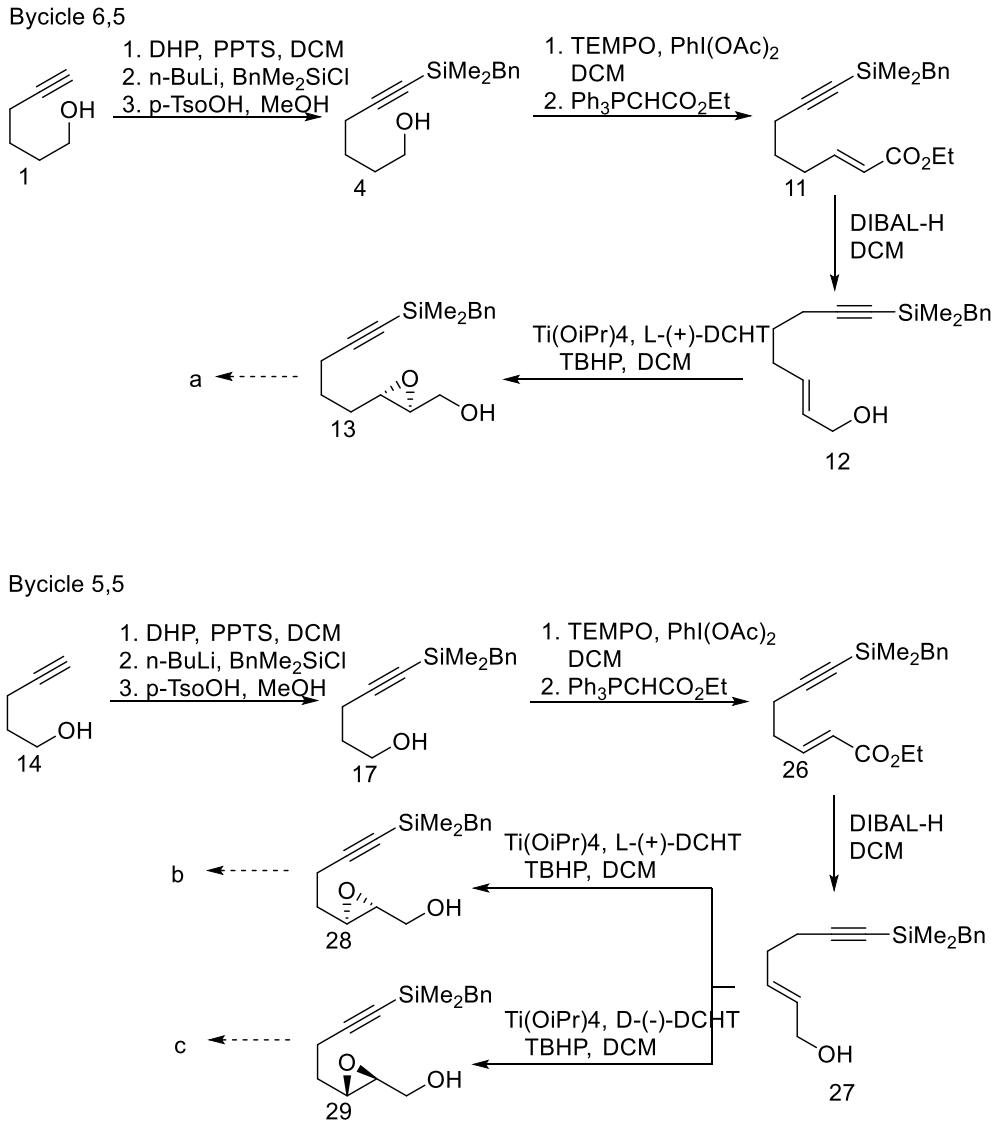


Fig. 13- Synthetic plan used in Santiago de Compostela to obtain the wanted precursors.

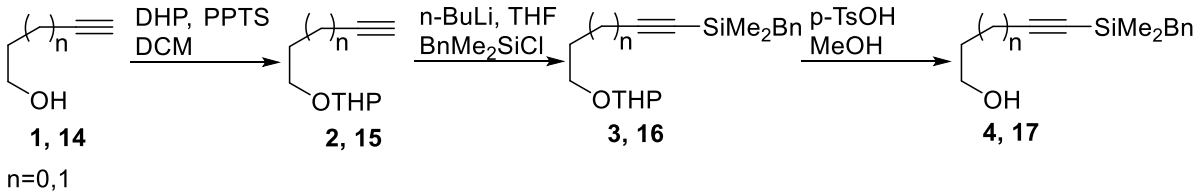
In this last method the starting materials were the same, the alcohols 5-hexyn-1-ol and the 4-pentyn-1-ol, **1** and **14**. Like in the other method, and for the same reasons, the alcohols were first protected, **4** and **17**. Then the compound **4** undergone a one-pot reaction of oxidation followed by a Wittig reaction which formed the **11** ester. The ester **11** was reduced with DIBAL-H, to form the corresponding allylic alcohol. The next step would be a Sharpless reaction to form the compound **13**.

3. Work done in Porto

3.1. Synthesis of the alcohols 4 and 17

The protection of the commercial compounds is needed to lower the volatility of these alcohols, and so increasing the yield. The use of this protecting group in particular (the benzyldimethylsilane) and the effects in the volatility of these compounds was studied before by our research group.

In order to obtain the two wanted alcohols, it was used the commercial compounds 5-hexyn-1-ol and 4-pentyn-1-ol. First, we had to protect the hydroxyl group, to become possible the protection of the triple bound. The hydroxyl group was first protected with dihydropyran in the presence of pyridinium p-toluenesulfonate and dichloromethane as solvent. Then was necessary a strong base to deprotonate the triple bond and becoming available for the bond of the protecting group. As protecting group was used benzyldimethylsilane. This protection reaction was done in THF and at -78°C. Then the protected alcohol was deprotected using a catalytic amount of p-toluenesulfonic acid, this reaction was done in the presence of methanol[62], Scheme 16.



Scheme 16- Protection of the alcohols 1 (n=1) and 14 (n=0) to obtain the alcohols 4 (n=0) and 17 (n=1).

The total yield obtained for the synthesis of these two alcohols was high, for the **4** the yield was 104 % and for the **17** was 85%. The observed high yield for the alcohol **4** can be justified by the existence of solvent. These two alcohols are very volatile, so we didn't concentrate that much, to ensure that the compound wasn't lost.

These compounds were then identified by NMR (^{1}H, ^{13}C and DEPT).

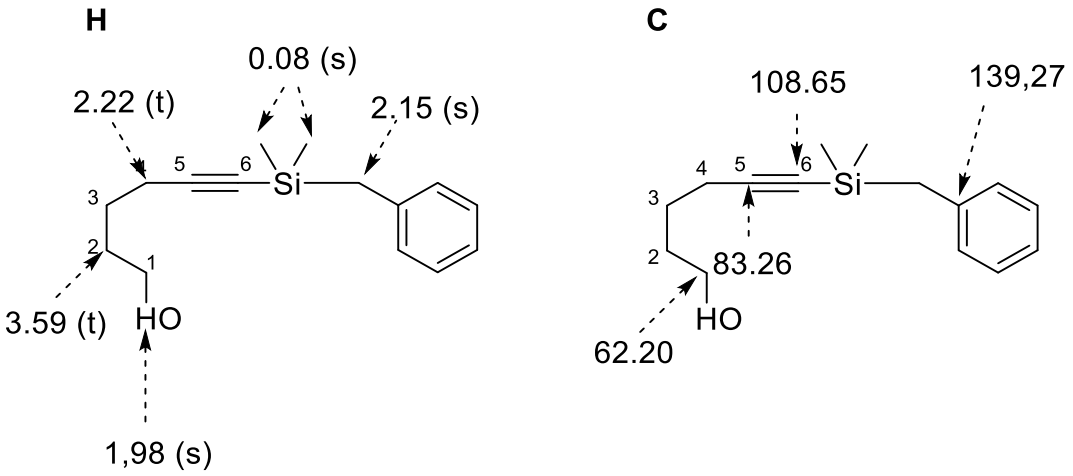


Fig. 14- Chemical shifts(ppm) for the compound 4. ^{1}H NMR- H and ^{13}C NMR-C.

By the analysis of the proton spectrum of the compound **4**, we can see the existence of some peaks, such as the chemical shift at 0.08 ppm that corresponds of the two methyl's attached to the silane group. The existence of this peak and the peaks that correspond to the phenyl group 7.20 – 7.03 ppm show that the protection of the triple bond was achieved.

By the analysis of the carbon spectrum we can see the peaks that correspond to the triple bond at 108.65 and 83,26 ppm and the peak that corresponds to the carbon of the phenyl group with a chemical shift of 139.27 ppm.

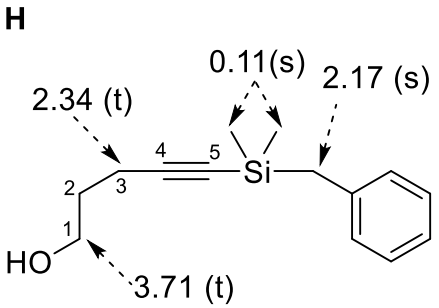
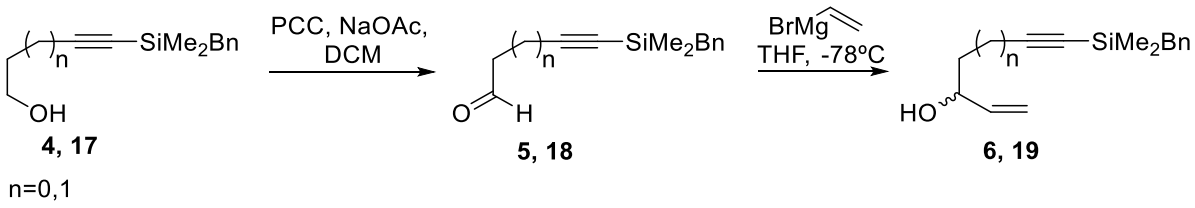


Fig. 15- Chemical shifts(ppm) for the compound 17. ^{1}H NMR- H.

By the analysis of the proton spectrum of the compound **17** we can see the same chemical shift, of the compound **4**, at 0,11 ppm corresponding to the two methyl's attached to the silane group, and by the analysis of the all spectrum, all the protons are identified showing that the protection of this molecule, was also achieved.

3.2. Synthesis of the alcohols 6 and 19

In order to synthetize the two alcohols **6** and **19** we first oxidized the alcohols **4** and **17** to obtain the aldehydes **5** and **18**. To oxidase the alcohols were used pyridinium chlorochromate [63] and sodium acetate. The obtained **5** and **18** aldehydes were alkylated using a Grignard reagent, Vinylmagnesium Bromide, to obtain the alcohols **6** and **19**[64], Scheme 17. This last reaction was done using as solvent THF and at -78°C.



Scheme 17- Reactional steps for the synthesis of the 6 (n=1) and 19 (n=0) alcohols.

For these two reactions the overall yield was 67 % for the **6** alcohol and 71% for the **19** alcohol.

The obtained products were then identified by NMR (^{1}H, ^{13}C and DEPT).

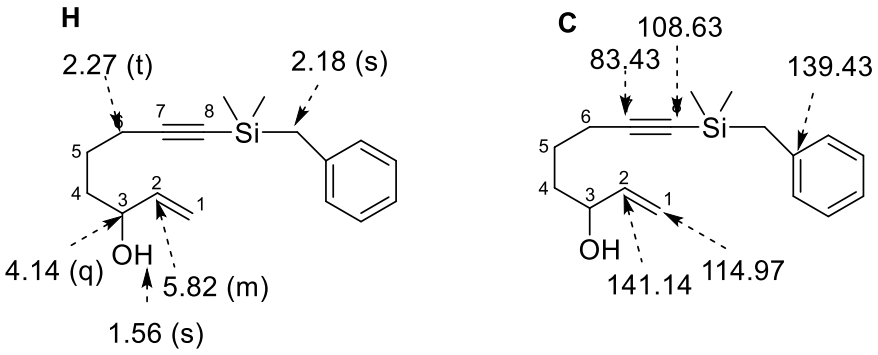


Fig. 16- Chemical shifts(ppm) for the compound 6. ^{1}H NMR- H and ^{13}C NMR-C.

By the analysis of the proton spectrum of the compound **6**, the most important peaks that show that the compound 6 was obtained are at the chemical shift 5.82 ppm, and 5.24-5.13 ppm, corresponding to the new double bond formed, positions C2 and C1 respectively.

By the analysis of the carbon spectrum we can see the peaks that correspond to the new double bond shows chemical shifts of 141,14 and 114,97 ppm. These peaks show that the compound **6** was formed.

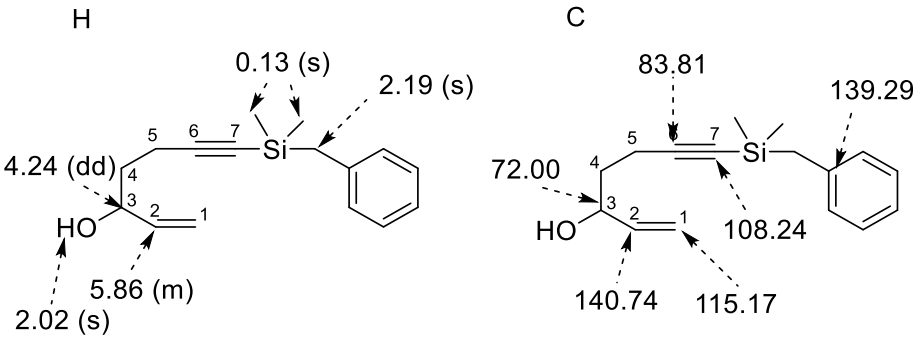


Fig. 17- Chemical shifts(ppm) for the compound 19. ^{1}H NMR- H and ^{13}C NMR-C.

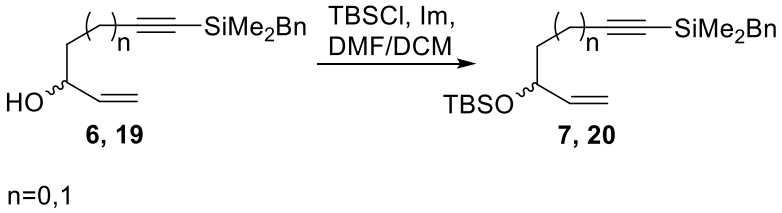
Similarly to the peaks found for the compound **6** we can identify by analysis of the proton spectrum of the compound **19**, the most important peaks that show that the compound **19** was formed, such as the peaks with a chemical shift of 5.86 ppm, and 5.27-5.15 ppm, that correspond to the new double bond formed, positions C2 and C1 respectively.

From the analysis of the carbon spectrum we can see the peaks that correspond to the new double bond shows chemical shifts of 140,74 and 115,17 ppm. These peaks show that the compound **19** was formed.

3.3. Synthesis of the protected alcohols 7 and 20

After the structures **6** and **19** were confirmed by the RMN, these alcohols were protected to be prepared for the next step, the Pauson-Khand reaction.

The two alcohols were protected with Tert-Butyldimethylsilyl chloride, and imidazole and using as solvent dimethylformamide in the reaction of the **6** and dichloromethane in the reaction of the **19**[65].



Scheme 18- Protection of the alcohols 6 (n=1) and 19 (n=0). In the protection of the alcohol 6 was used DMF and in the reaction of 7 was used as solvent DCM.

The yield obtained for the protection of these two alcohols is different. The yield of the protection of **6**, where it was used as solvent DMF, was 84% and the yield associated with the protection of **19**, using as solvent DCM, was 64%. This difference seems to be

associated with the solvent used, thus showing that for this type of protection DMF is the solvent of choice, like in the protocol used for this reaction[65].

These compounds were then identified by NMR (^{1}H, ^{13}C and DEPT).

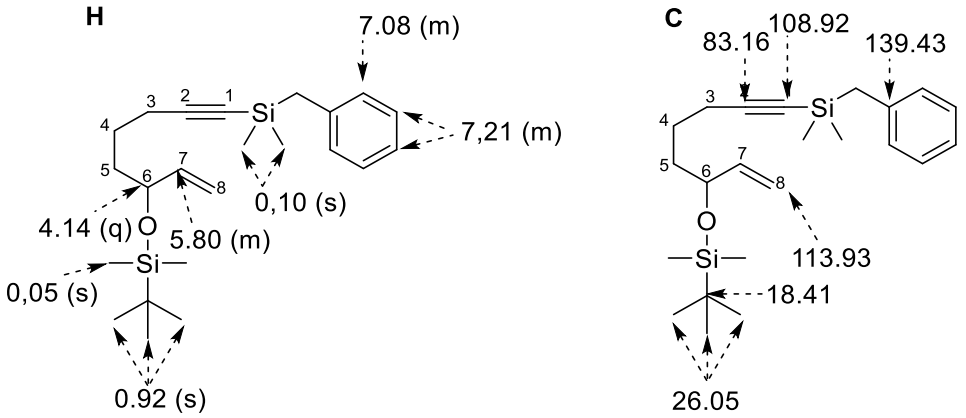


Fig. 18- Chemical shifts(ppm) for the compound 7. ^{1}H NMR- H and ^{13}C NMR-C.

By analysis of the proton spectrum of the compound **7**, the most important peaks that show that the alcohol was protected were the chemical shifts at 0,05 ppm, corresponding to the methyl in the silane group and the peak at 0.92 ppm corresponding to tert-Butyl group.

By the analysis of the carbon spectrum we can see the peaks that correspond to the protecting group, with chemical shifts at 18.41 ppm, that corresponds to the quaternary carbon, and 26.05 ppm that corresponds to the tert-Butyl group.

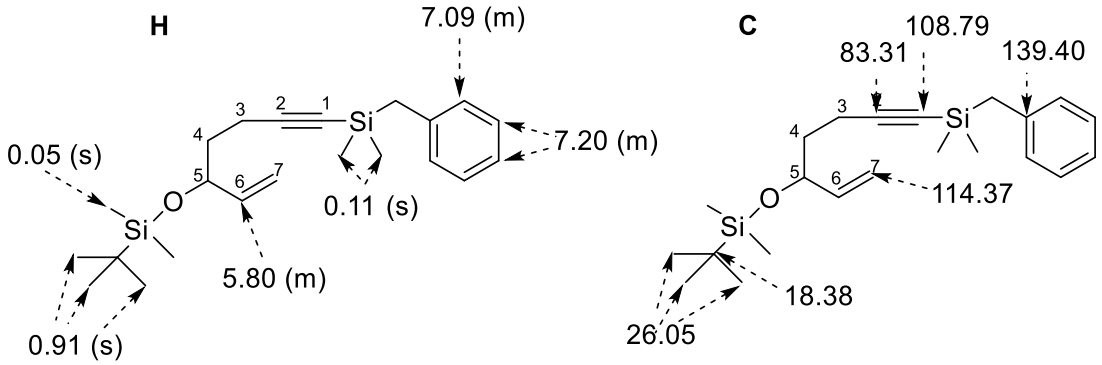


Fig. 19- Chemical shifts(ppm) for the compound 20. ^{1}H NMR- H and ^{13}C NMR-C

From the analysis of the proton spectrum of the compound **20**, the most important peaks that show that the alcohol was protected were the chemical shifts at 0,05 ppm,

corresponding to the methyl in the silane group and the peak at 0.91 ppm corresponds to the tert-Butyl group.

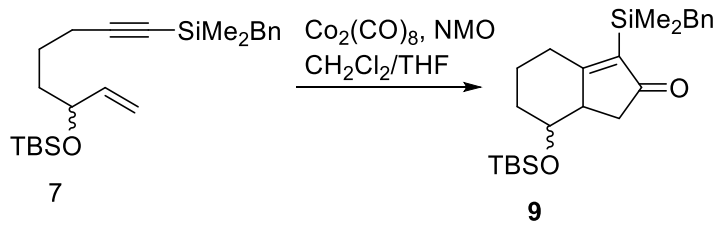
By the analysis of the carbon spectrum we can see the peaks that correspond to the protecting group with chemical shifts at 18.38 ppm, corresponding to the quaternary carbon, and at 26.05 ppm that corresponds to the tert-Butyl group.

3.4. One of the strategies used for the resolution of the alcohol 7

One of the first strategies used to in order to separate the racemic mixture and get one of the stereoisomers wanted it was trying the separation of the compounds only after cyclization. First, we made the cyclization by using the Pauson-Khand reaction, then we reduced the obtained ketone to the wanted alcohol. This alcohol would then react with a chiral isocyanate which would allow the separation through silica column. This last part of the resolution was done in my research group [66]. However, this resolution attempt didn't work.

3.4.1. Synthesis of the ketone 9

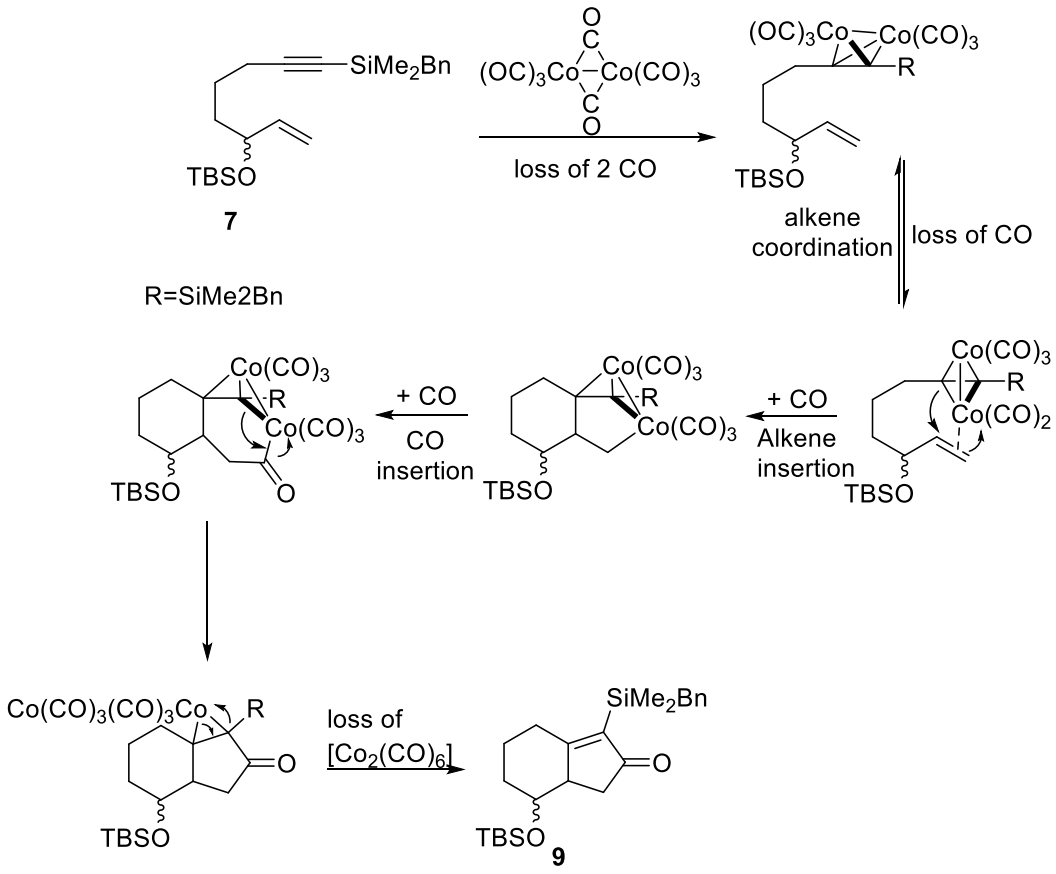
To obtain the ketone **9**, we used a Pauson-Khand reaction using as starting material the protected alcohol **7**. In order to make this reaction, we used the protocol from the Santiago research group[55]. The yield obtained for the Pauson-Khand reaction was 67%.



Scheme 19- Cyclization of the protected alcohol 7 to obtain the ketone 9.

The Pauson-Khand reaction was first discovered in 1973 by Ihsan U. Khand and Peter L. Pauson et all[67], and is the reaction of dicobalt octacarbonyl complexes with alkenes to form cyclopentenone. This reaction is characterized by a [2 + 2 + 1] cycloaddition in which a triple bond, a double bond and carbon monoxide form a cyclopentenone. So, this type of reaction leads to the formation of three new bonds and, in this case, two cycles since this reaction is a intramolecular version.

This type of reaction can be accelerated using promoters, in this case the promoter used was NMO. The promotors can facilitate the coordination of the alkene to the cobalt atoms. The mechanism proposed for this reaction is in the Scheme 20[68].



Scheme 20- Cyclization of the bicycle[68-69].

First occurs the formation of a complex with the alcohol **7**, in which the initial octacarbonyl complex loses 2 CO ligands. Then occurs the alkene coordination with this complex in which occurs the loss of a CO molecule. This step is the rate-determining step and in order to accelerate this process it is used promoters. Then the olefin is inserted into a Co–C bond forming a cobaltacycle. Then an insertion of a CO molecule occurs and a carbonyl group is formed. Finally, the release of dicobalt hexacarbonyl occurs, and the wanted ketone **9** is formed [70].

The obtained compound was then analyzed by NMR (^{1}H, ^{13}C, DEPT).

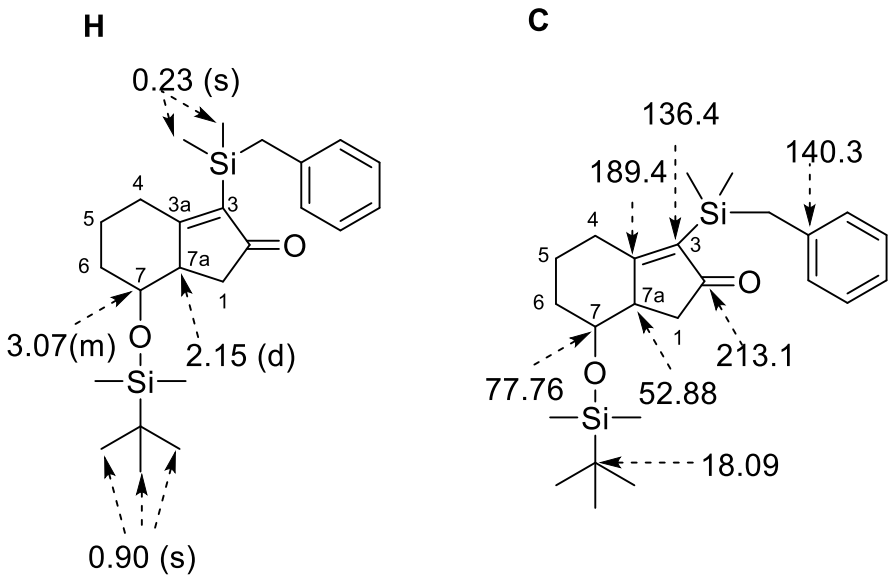


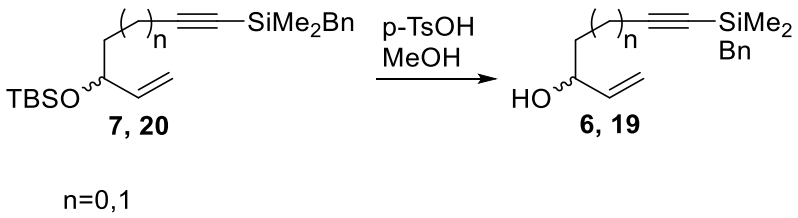
Fig. 20- Chemical shifts(ppm) for the compound 9. ^{1}H NMR- H and ^{13}C NMR-C

From the analysis of the proton spectrum of compound **9**, we can see some peaks that correspond to hydrogens in the bicyclic ring and to the peaks that correspond to the protecting group.

From the analysis of the carbon spectrum it is possible to identify the peaks that correspond to the used protecting groups. It is also possible to observe the chemical shifts of the carbons in the bicyclic ring, such as two quaternary carbons at the position 3a and 3 with a chemical shift of 189.4 ppm and 136.4 ppm respectively. The other important peak is the one that corresponds to the carbonyl at position 2, with a chemical shift of 213,1 ppm.

3.5. Deprotection of alcohols 6 and 19

Since we didn't succeed in our attempt to perform the quiral resolution the molecules using an isocyanate, we deprotected the remaining alcohols **7** and **20**. This deprotection was done using a catalytic quantity of *p*-TsOH in methanol.



Scheme 21- Deprotection reaction to obtain the alcohols 6 and 19. 6 (n=1), 19 (n=0).

This deprotection had a yield of 89% for the **19** alcohol. The **6** had a yield too big, above 100%, which means that it should have solvent, can be explained by the fact that this deprotection didn´t needed purification, the TLC showed only one spot.

These compounds were identified by NMR (^{1}H, ^{13}C and DEPT).

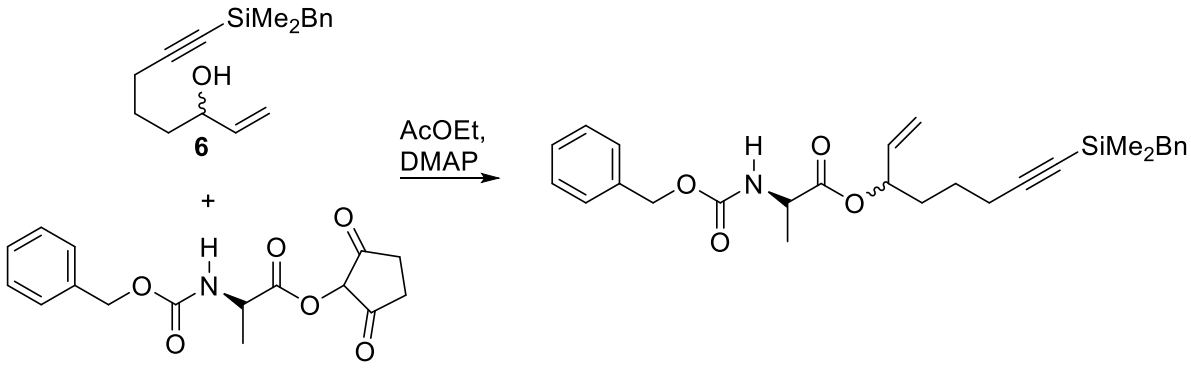
The spectra from these two compounds were similar to the presented in the section 3.2.

3.6. Chiral resolution

We attempt to perform the resolution of the obtained alcohols using another two resolving agents. Then we tried it using enzymes (Novozyme 435, and Amano enzymes). Our last attempt of resolution used a Sharpless asymmetric epoxidation.

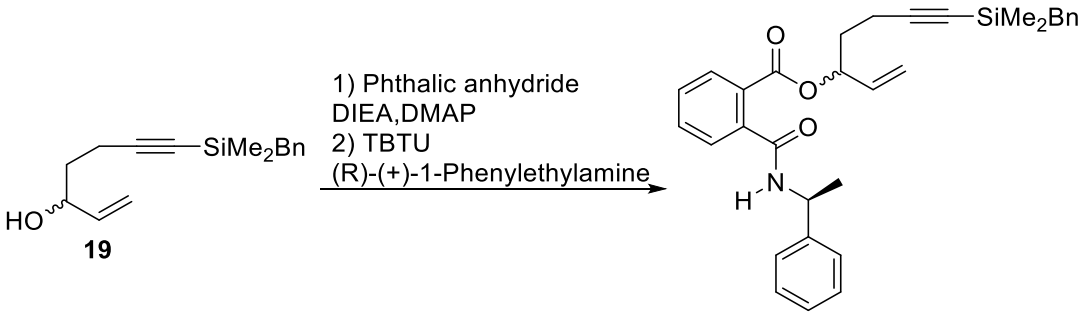
3.6.1. Resolution using resolving agents

In this attempt we esterified the compound **6** with Z-L-alanine hydroxysuccinimide ester to form the wanted diastereomers, scheme 22. This resolution method didn't work.



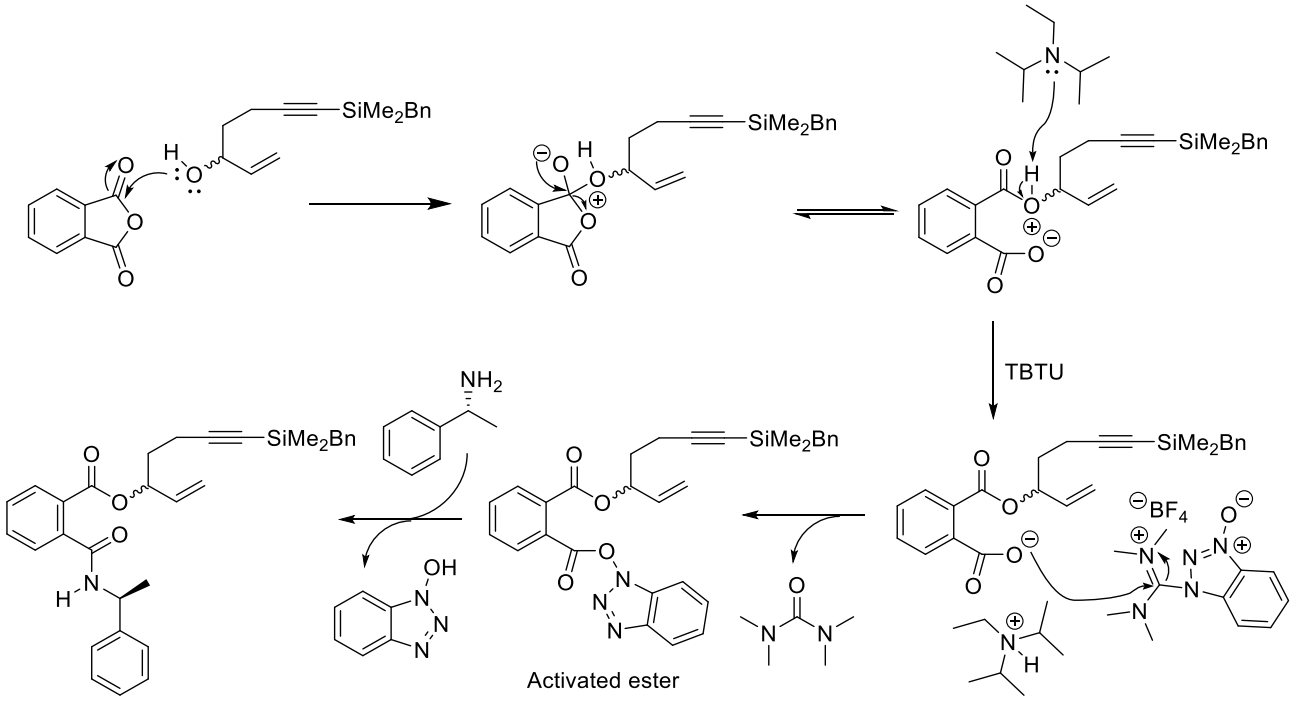
Scheme 22- Attempt of alcohol 6 resolution using z-Ala-OSu.

The other method used is shown in the Scheme 23. In this attempt we tried to make a diastereomer with a more common rigid structure, and thus, making the alcohol easier to separate. In this strategy we used, as the rigid structure the phthalic anhydride. That would be esterified with the alcohol **19**, and then using the chiral reagent, (R)-(+)-1-Phenylethylamine, the diastereomers should be obtained



Scheme 23- Attempt of alcohol 19 resolution.

The proposed mechanism for this reaction is shown in Scheme 24.



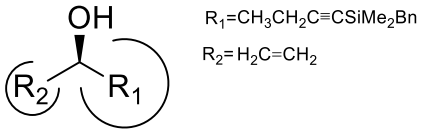
Scheme 24 - Mechanism used to esterify the alcohol in order to obtain diastroreoisomers.

In the mechanism the alcohol attacks the phthalic anhydride in the carbonyl group, which will lead to an opening of the anhydride. Then the base, in this case the DIEA, takes the proton of the alcohol. With the addition of the TBTU to the reaction, an activated ester is formed, that will then react with the chiral molecule added, in this case the (R)-(+)-1-phenylethylamine.

This reaction didn't work. We saw some spots in the TLC, but after separation and RMN analysis we concluded that this reaction didn't worked.

3.6.2. Resolution method using different enzymes

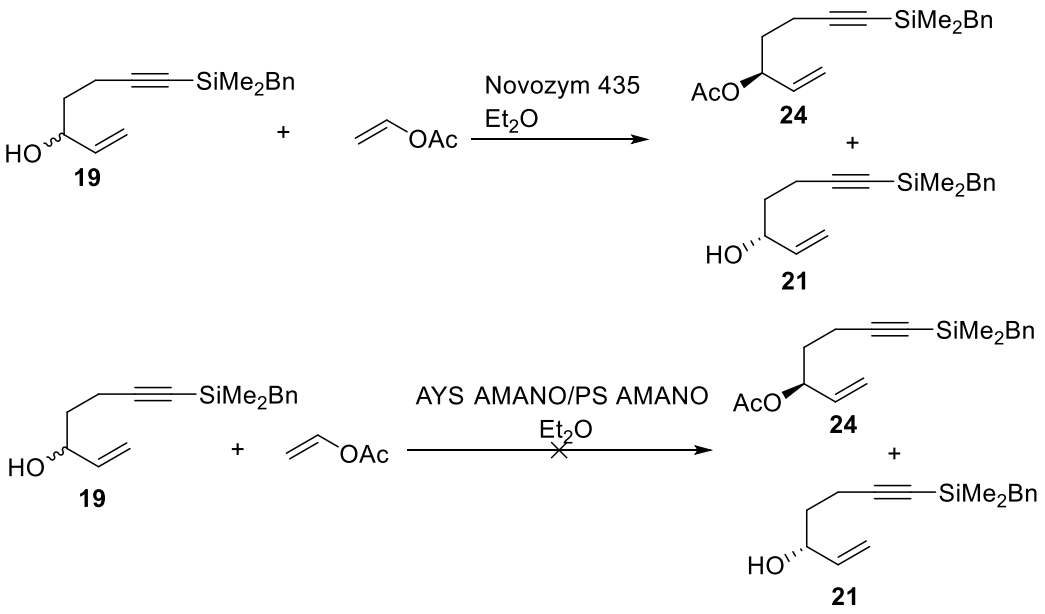
The kinetic resolution has been used as a green alternative when compared with the others resolution methods. Enzymes show a high enantioselectivity towards an ample range of secondary alcohols. This enantioselective activity can be used to predict which is the enantiomer of a secondary alcohol that reacts faster in a lipase catalyzed reaction, see Scheme 25. These reactions can be performed with high regio- and enantioselectivity under mild reaction conditions[72].



Scheme 25- Enzyme binding regions.

The characteristic enantioselectivity found with lipases occurs because lipases display alcohol binding regions with a larger hydrophobic pocket and a medium one. So only one of the enantiomers will have a better fit in the binding region, and so, only one of the enantiomers will react[73].

The enzyme resolution method was done using three different enzymes. The first one was done with Novozyme 435. The other two attempts were done with two different AMANO enzymes, the PS and the AYS. The reaction used for these three enzymes was the same and performed with the same quantities and solvent. The reaction catalyzed by the three enzymes was the transterification of the S-enantiomer with ethenyl acetate (Scheme 26). These procedures were done according to the literature[71].



Scheme 26 - Attempt of alcohol 19 resolution using three different enzymes, Novozym 435, AYS AMANO and PS AMANO.

After 35 hours of reaction, only with Novozyme 435 could be found the wanted product. With the other two enzymes we could not observe the formation of any product.

The compounds obtained in the reaction with the Novozyme 435, were analyzed first by NMR (1H, 13C, DEPT).

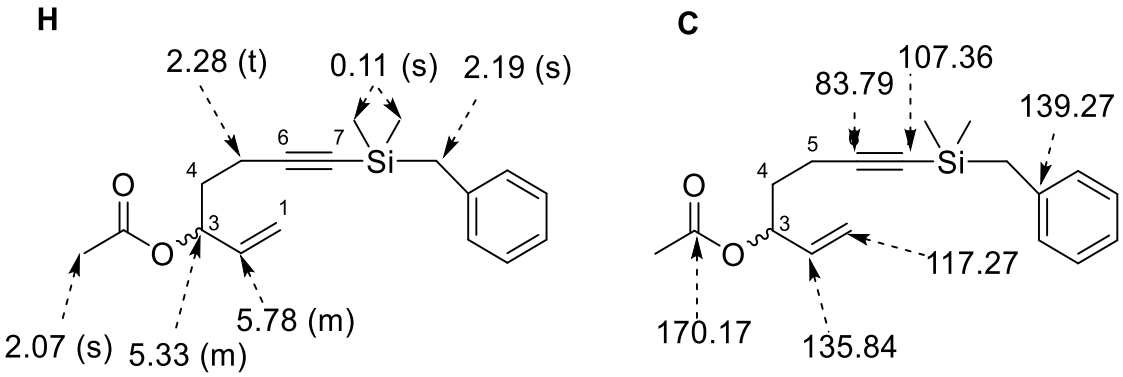
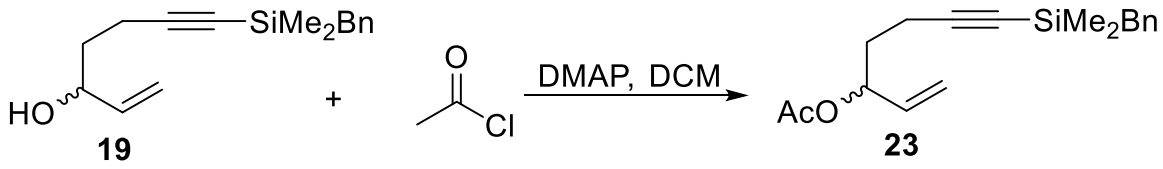


Fig. 21- Chemical shifts(ppm) for the compound 23. ^{1}H NMR- H and ^{13}C NMR-C

The analysis of the proton spectrum of the compound **23**, showed that the alcohol was acetylated, with a chemical shift at 2,07 ppm, corresponding to the methyl of the acetyl group, and a peak at 5,33 ppm corresponding to the hydrogen at C3.

From the analysis of the carbon spectrum we could confirm the acetylation of the alcohol, with a peak at 170.17 ppm corresponding to the carbonyl group.

In order to conclude if the alcohol resolution occurred, we analyzed the mixture of compounds by HPLC using a chiral column. Therefore, we needed to synthetize first the racemic acetylated alcohol by the reaction shown in Scheme 27. Using the same starting alcohol **19** and acetyl chloride to esterify the alcohol in the presence of DMAP.



Scheme 27- Esterification reaction of the **19** alcohol.

This reaction had a yield of 89%. The compounds were then analyzed by NMR (^{1}H,^{13}C, DEPT), and the spectra obtained was similar to the one obtained with the enzymatic catalyze.

After the synthesis of **23**, we had to find the eluent conditions that were able to separate both enantiomers. The conditions were studied with the racemic mixture of the acetylated alcohol, **23**. Then, after we had been able to get the chromatogram peaks well resolved (using a eluent of 99% of hexane and 1% of isopropanol and a flow of 0,5 mL/min) we used the same conditions to run a sample with the acetylated compound obtained from the reaction catalyzed by the enzyme. The chromatograms are displayed in the Fig. 22 and Fig. 23.

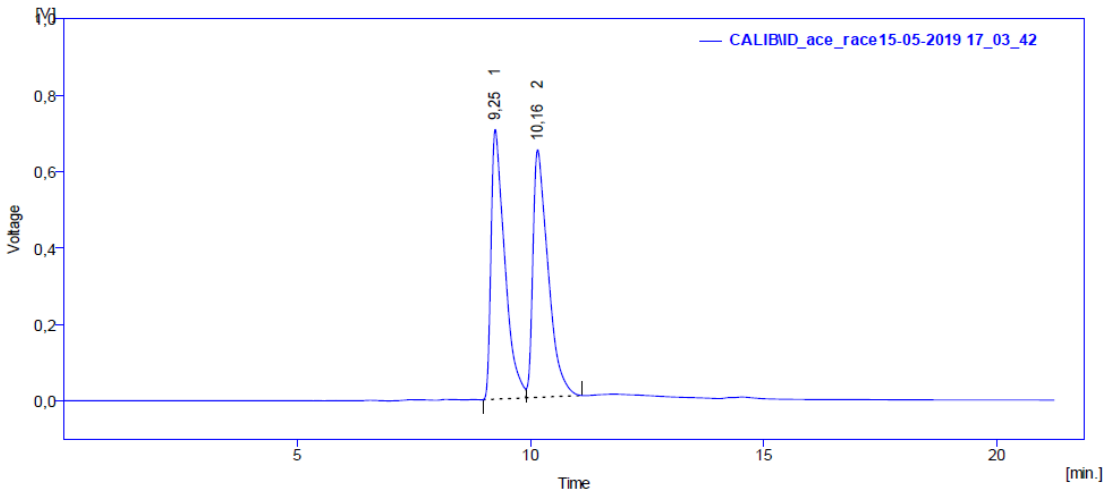


Fig. 22- Chromatogram obtained for the racemic acetylated alcohols.

Peak	Retention time [min]	Area	Area [%]
1	9,252	14379,146	49,5
2	10,162	14643,605	50,5
	Total	29022,751	100,0

Table 1- Data obtained by the integration of the two peaks of the chromatogram above.

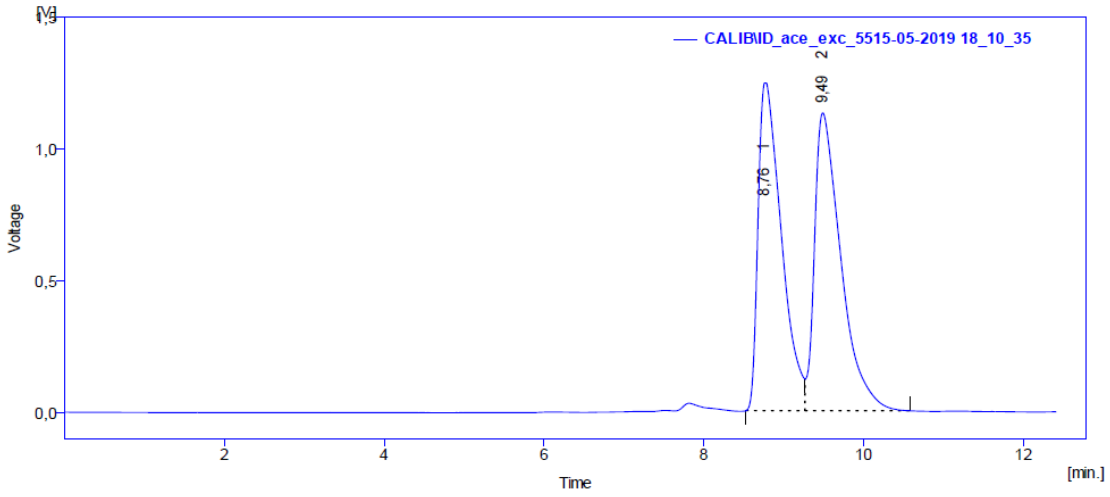


Fig. 23- Chromatogram obtained for the alcohol acetylated by the Novozyme 435

Peak	Retention time [min]	Area	Area [%]
1	8,760	25186,133	49,2
2	9,490	25998,061	50,8
	Total	51184,194	100,0

Table 2- Data obtained by the integration of the two peaks of the chromatogram above.

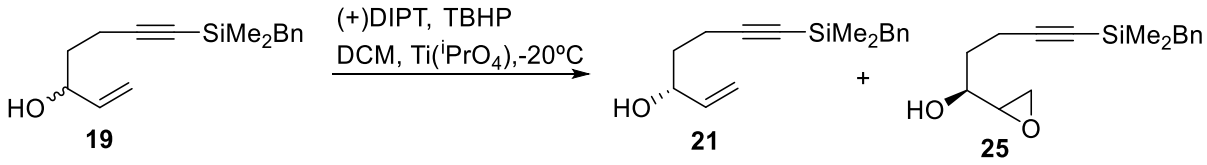
Observing the chromatogram in the Fig. 22 and the corresponding table we can see two peaks with similar area values. Each peak correspond to an enantiomer, as is expected, since the mixture is racemic.

Observing the chromatogram in the Fig. 23, when the reaction was catalyzed by Novozyme 435, we can also see two peaks with similar areas, which was not expected. So, we can conclude that this resolution didn't work. The chromatogram obtained for the acetylated alcohol obtained in the reaction catalyzed by the Novozyme 435 showed two peaks which means that the enzyme catalyzed the reaction of both enantiomers.

3.7. Resolution method using a Sharpless asymmetric epoxidation

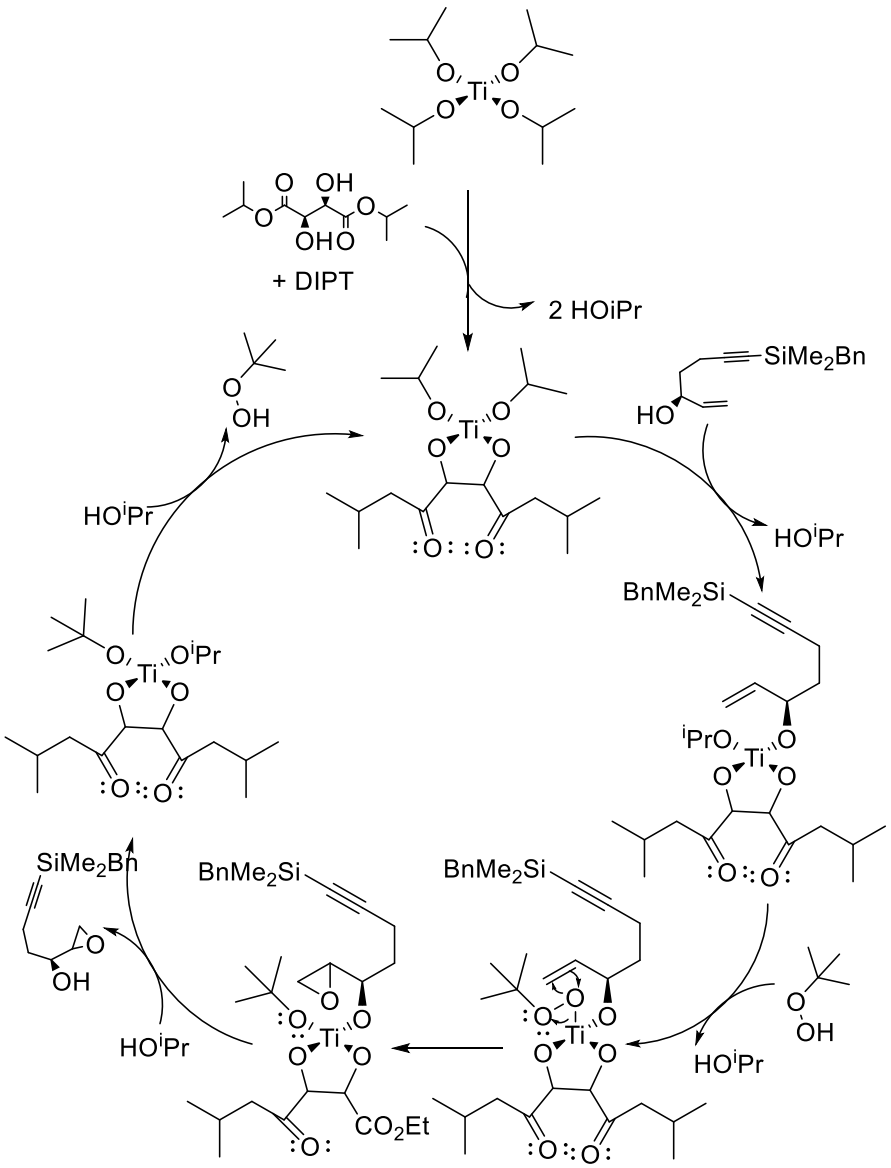
This was the last resolution method attempted. We did this epoxidation using as the start material the **19** alcohol, and we followed the literature procedure[55, 74].

This last method is known as the Sharpless asymmetric epoxidation. This reaction was first reported by K.B. Sharpless and T. Katsukib in 1980 and is characterized by the $Ti^{(IV)}$ alkoxide-catalyzed epoxidation of chiral allylic alcohol using a chiral tartrate ester and an alkyl hydroperoxide as an oxidant. This reaction is known for the high yields and excellent enantiomeric excess [75].



Scheme 28- Resolution of the alcohol 19 using the Sharpless asymmetric epoxidation method.

This reaction was done with an already chiral alcohol **19**, so this process was necessary only to separate the enantiomers. The epoxidation reaction only occurred with one of the enantiomeric alcohols, in this case the R alcohol. Since the other alcohol **21,** doesn't react, the separation through a chromatographic column is viable. The (R) alcohol only, reacts with the L-(+)- diisopropyl tartrate[74]. The mechanism of this reaction is shown in the Scheme 29.



Scheme 29- Mechanism for the Sharpless asymmetric epoxidation.

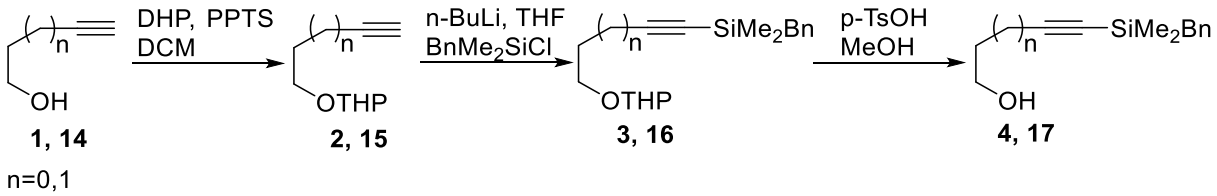
The first step in this reaction is the exchange of the ligands of $Ti(O^i\text{-}PrO)_4$ with the Diisopropyl tartrate. After this first complex formation, this will undergo more ligands exchange, first with the alcohol that was reacting and then with the tert-Butyl hydroperoxide. After the alcohol being epoxidized, it is released to the solution and then TBHP is released from the complex to the solution and the first complex is formed again[75-76].

We were not able to confirm this resolution method, we were only able to analyze the NMR (^1H, ^{13}C and DEPT) spectra. We still need to analyze by HPLC, using a chiral column. The obtained alcohol has the same NMR as the one in the section 3.2.

4. Work done in Spain

4.1. Synthesis of the alcohols 4 and 17

The reactional steps for the protection of these two alcohols were the same used in Porto. The principal difference is that at the University of Santiago we did this reaction using higher amounts of the commercial alcohols 5-hexyn-1-ol and 4-pentyn-1-ol. In order to ensure that we had enough compound for all the steps needed to synthetize the three analogs. So, we started with 15 grams of 5-Hexyn-1-ol and 20 grams of 4-Pentyn-1-ol. We had some difficulties in the purification of this amounts because of the very long time needed for the purification. The reaction of the protection of the triple bond were performed twice for each alcohol.

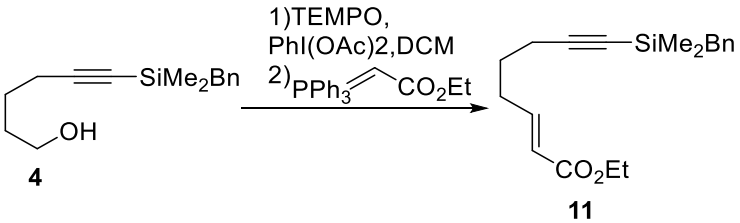


Scheme 30- Reactional steps for the synthesis of the alcohols 4 and 17 (4 for n=1 and 17 for n=0).

Due to the high volatility of the products and due to the high quantities of products we didn't concentrate till the compound was dried, in order to ensure the minimum lost product possible. Moreover, we only deprotected compound **3**, thus maintaining the **16** protected. We only used the compound **4** in the next reactions.

4.2. Synthesis of the ester 11

The ester **11**, was synthetized using as starting material the alcohol **4**, with a one pot reaction, in which the alcohol was first oxidized using TEMPO and then the formed aldehyde was esterified using a Wittig reaction.



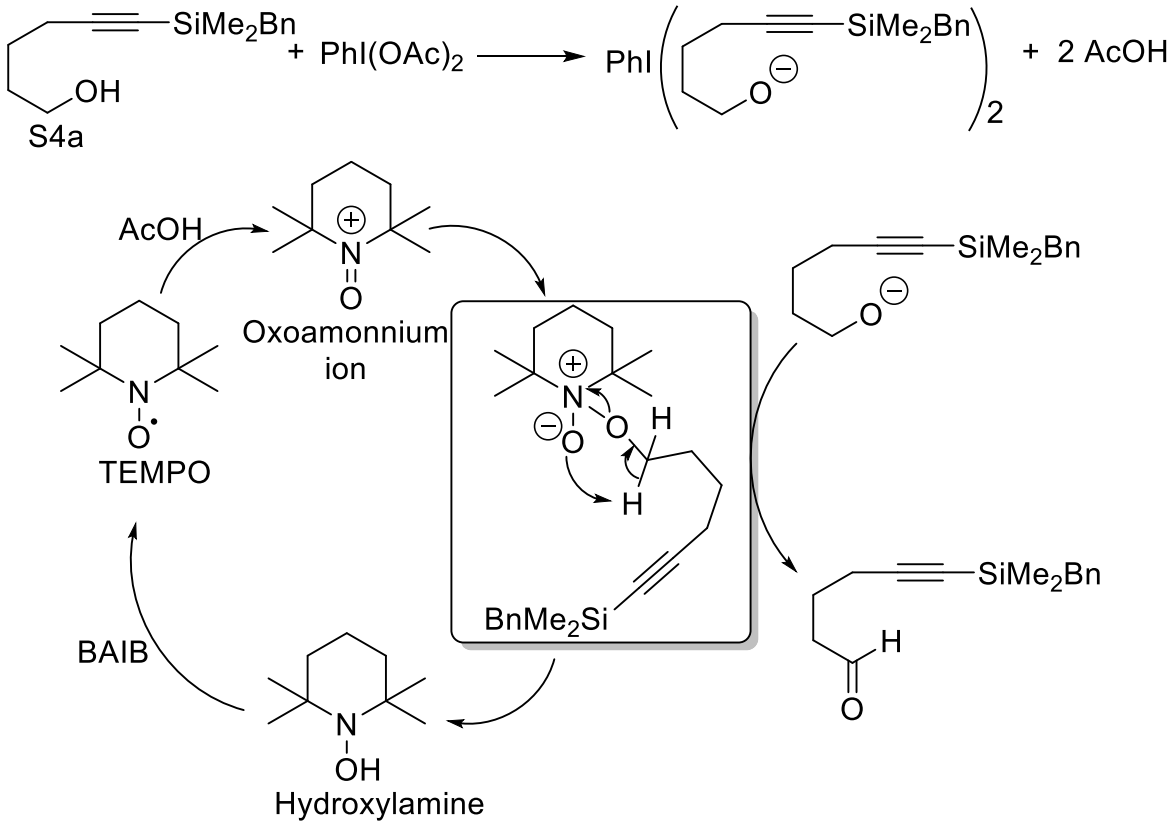
Scheme 31-Oxidation of 4 alcohol and Wittig reaction to form the ester 11.

This reaction had a yield of 83%:

4.2.1. TEMPO oxidation

In the TEMPO oxidation it is used a catalytic quantity of TEMPO, 2,2,6,6-tetramethyl-1-piperidinyloxyl, combined with diacetoxyiodobenze (BAIB), this reaction can be done at room temperature and using as solvent DCM. Under these conditions the primary alcohols can be rapidly oxidized to aldehydes without any noticeable overoxidation to carbonyl compounds, thus showing high selectivity. The oxidation reaction of primary alcohols to aldehydes is fast, taking about 2 hours[77].

In this case the primary alcohol **4** was oxidized to the corresponding aldehyde. In the Scheme 32 is presented the proposed mechanism for this oxidation.

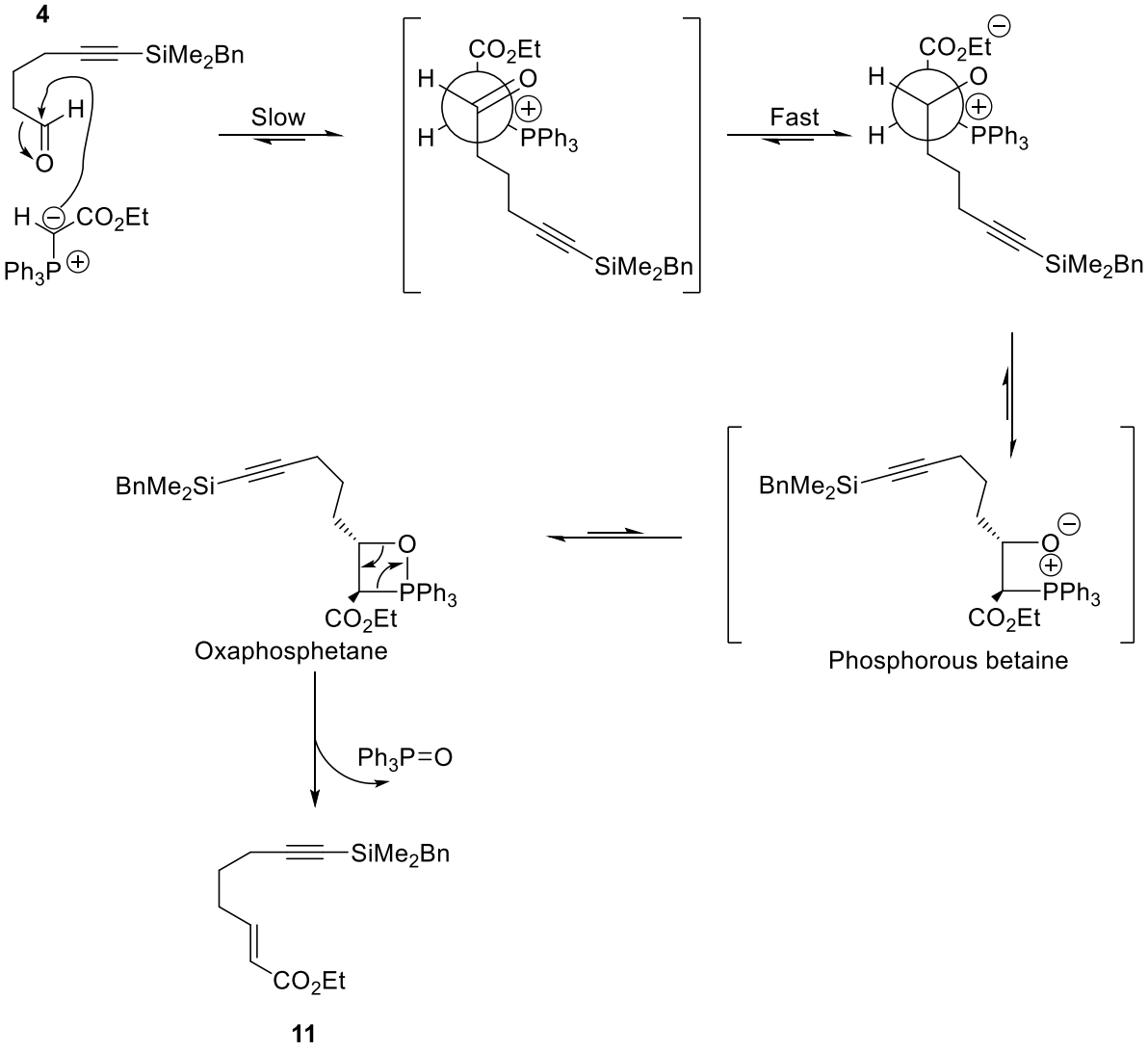


Scheme 32- Proposed mechanism for the oxidation of alcohol 4 using TEMPO[77-78].

In this mechanism there is a ligand exchange between the alcohol and the BAIB, this exchange leads to the production of acetic acid. This acetic acid will transform TEMPO in an oxoammonium ion. Then, the oxidation of the wanted alcohol occurs. When this oxidation occurs, the oxoammonium is reduced to hydroxylamine and the alcohol is oxidized to the wanted aldehyde. After this process BAIB comes to regenerate TEMPO in order to close the catalytic cycle[77].

4.2.2. Wittig reaction

In 1950 G..Wittig and G. Geissler concluded various studies in which they reacted various aldehydes and ketones with several phosphoranes to obtain the corresponding olefins. This reaction consists in the formation of carbon-carbon double bonds (olefins) from the carbonyl compounds and the phosphoranes [79].



Scheme 33- Proposed mechanism for the Wittig reaction.

In the Wittig reaction the active reagent is the phosphorous ylide, see Scheme 33. The stabilized ylide, used in this reaction, reacts with the carbonyl group of the aldehyde in a reversible and slow step. This step will form the intermediary betaine. Then the oxygen attacks the phosphorous giving rise to another intermediate the oxaphosphetane. This last intermediate then breaks down and forms the corresponding E-olefin, **11**[80]. In this case the reaction occurred with an aldehyde, which is characterized as a faster reaction. In this

reaction the only groups that can react are ketone and aldehydes, the other groups stay intact during the reaction. In this reaction we used a stabilized ylide, that forms predominantly E-olefins with the aldehydes. The non-stabilized ylides give predominantly Z-olefins[79].

After reaction, the compound obtained was identified by NMR (1H,13H, DEPT), Fig. 24.

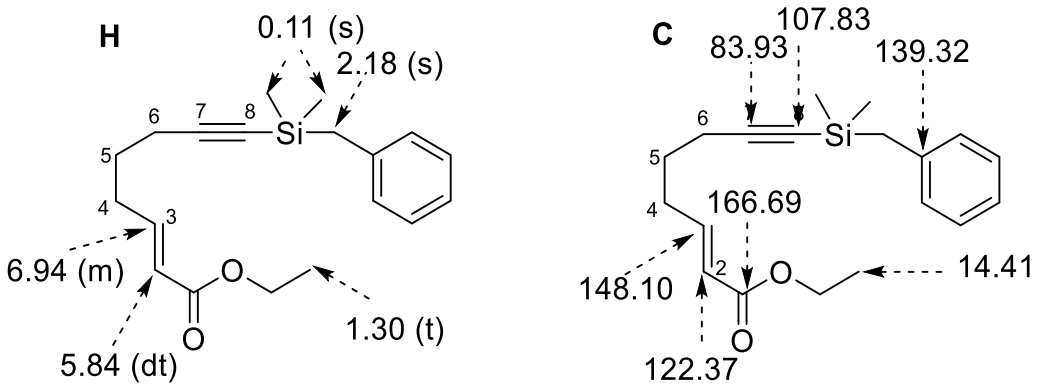


Fig. 24- Chemical shifts(ppm) for the compound 11. ^{1}H NMR- H and ^{13}C NMR-C

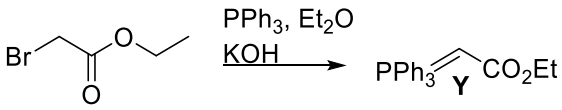
From the analysis of the proton spectrum of the compound **11** we observed, the most important peaks that show that this compound was formed have chemical shifts of 5,84, this peak corresponds to the double bond at C2 and the chemical shift at 1,30 ppm that corresponds to the methyl group.

By the analysis of the carbon spectrum we can see some peaks that confirm the formation of the compound **11** such as the chemical shift at 166,69 ppm, that corresponds to the carbonyl group.

4.2.2.1.Phosphorous ylide synthesis (Y)

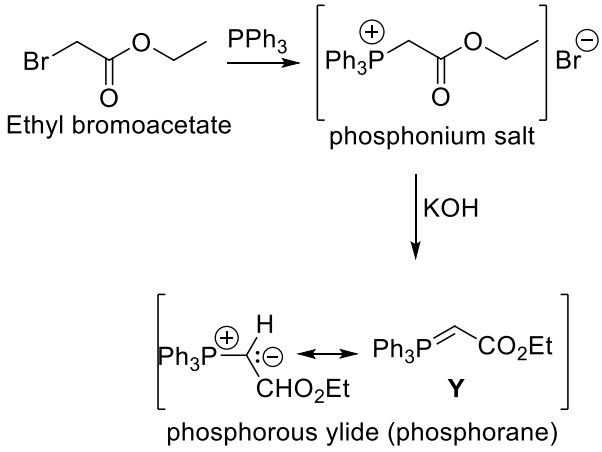
The ylide needed for the Wittig reaction was synthetized in Porto. This reaction was prepared with ethyl bromoacetate and triphenylphosphine.

This synthesis was improved in Porto by our research group. Before the reaction needed to be performed in toluene and stayed in reflux for 10 hours. The yield of this reaction was 60%[81]. However, using our new approach, this synthesis is done at room temperature, and as solvent we use Et_2O. The yield obtained in this synthesis was 96%.



Scheme 34- Ylide synthesis, new approach.

The mechanism for this synthesis is presented in the Scheme 35.



Scheme 35- Phosphorous ylide formation.

Firstly, when the ethyl bromoacetate is mixed with the triphenylphosphine occurs the formation of the corresponding phosphonium salt. Then when the base is added, occurs the deprotonation of the phosphonium salt which will form the phosphorous ylide[79].

This compound was identified by NMR (^{1}H, ^{13}C and DEPT).

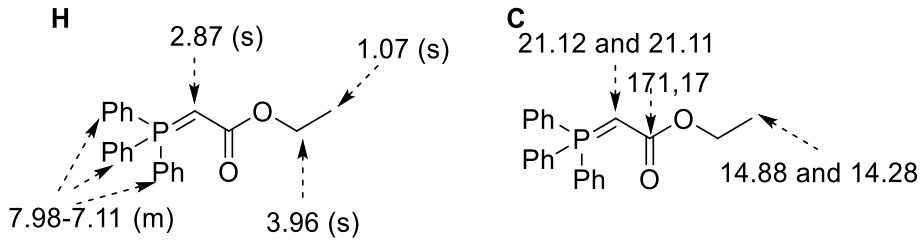


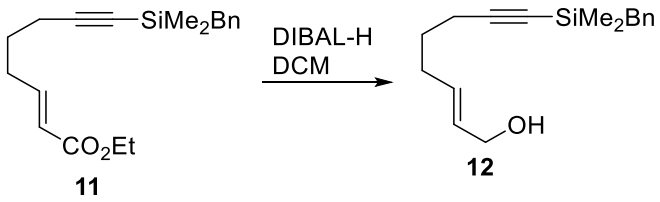
Fig. 25- Chemical shifts(ppm) for the compound Y. ^{1}H NMR- H and ^{13}C NMR-C.

From the analysis of the proton spectrum, we can see the existence of some peaks with a chemical shift between 7,98 and 7,11 ppm, that by integration show a value of 15. These peaks correspond to the three phenyl groups that are attached to the phosphor atom. Other important peak has a chemical shift of 2,87 ppm. This peak corresponds to the proton of the double bond.

From the analysis of the carbon spectrum we can see the peaks that correspond to the double bond, 21,12 and 21,11 ppm, and the peak that corresponds to the carbonyl group, that shows a chemical shift of 171,12 ppm.

4.3. Reduction to obtain the alcohol 12

In order to reduce the ester **11** to the alcohol **12**, we used DIBAL-H. In this reaction is necessary to use 2 equivalents of DIBAL-H. The first equivalent is used to form the aldehyde and the second equivalent is used to form the wanted alcohol, **12**. The yield obtained for this reaction was 54%.



Scheme 36- Reduction of the ester 11 to the alcohol 12.

The compound was later identified by NMR (^{1}H, ^{13}C and DEPT).

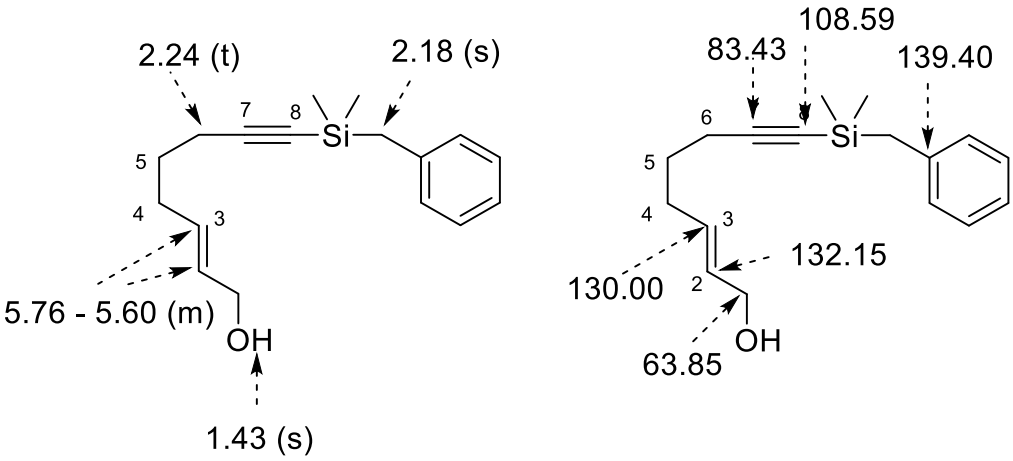


Fig. 26- Chemical shifts(ppm) for the compound 12. ^{1}H NMR- H and ^{13}C NMR-C.

By analysis of the proton spectrum of the compound **12**, the most important peak that show that this compound was formed have chemical shift of 1.43 ppm and corresponds to the hydrogen of the hydroxyl group. The other important characteristic that showed the formation of the alcohol **12** is the lack of some of the peaks that correspond to the molecule **11**.

By the analysis of the carbon spectrum we can see some peaks that confirm the formation of the compound **12** such as the chemical shift at 63.85 that corresponds to the carbon at the position 1.

Discussion and conclusions

In this work we studied two routes to obtain the same precursors of the enantiomerically pure CD bicyclic rings. The first route started but not finished was done in Spain. The other route was done in Porto.

The first route was already started for one of the analogs and showed to be promising. Here we follow this route but with higher great amounts of initial reagents. Due to time constraints the final precursors were not obtained.

In Porto we tried some resolution approaches to obtain the enantiomerically pure CD bicyclic ring, but most of them failed, the only one that seems to work was the Sharpless epoxidation, but we are still waiting for the results. In Porto we wanted to find a more practical, faster and green resolution method, since the route developed in Spain showed some problems when done with higher amounts of reagents. The route developed in Spain needed a lot of purifications and had several steps before getting to the bicyclic ring, which was unfeasible because of the high amount off compound needed to follow the whole analog synthesis.

In the route done in Spain the resolution would been done with a Sharpless epoxidation reaction. In this reaction, in opposite to the Sharpless reaction done in Porto, since the alcohol was a primary alcohol, the reaction would form only one product an epoxide with the correct configuration. This way is good to obtain the compound A. Since we only wanted the compound with only a configuration.

In the Sharpless epoxidation done in Porto, the reaction occurred with a secondary alcohol, here we could only separate the alcohols. This reaction is much slower. And only one of the enantiomers reacts forming the epoxide. This reaction has a low yield compared with the Sharpless reaction with the primary alcohol. This reaction is better and more efficient for the synthesis of the analogs B and C. Since we needed both enantiomers. The bigger problem with this method is that the Sharpless reaction needs to react for 21 days, which is a long time. The other problem in this method is that we would obtain an alcohol with one configuration ready to cyclize and an epoxide with the other configuration, this would need to be transformed in the alcohol pretended to be able of cyclize.

Future perspectives

In order to make the synthesis of these analogs possible is important to find another way of resolution that is faster and that have less steps to form the wanted bicyclic ring. This would lead to minimum loss of compound synthesized, since to get to the analog is important to have the amounts needed for the biological studies.

So is needed further studies and more time to continue and finish this work.

Experimental procedure

1. Materials and methods

All sensitive water reactions were done under argon. Glassware used in these reactions were previously dried at 100°C, and then were purged with argon. Solvents were distilled under argon, using the corresponding drying agents. DCM was dried with P_2O_5 and the THF was dried with potassium. Other solvents used were dried with molecular sieves 4Å.

The solution of ^{n}BuLi (dissolved in Hexane, Aldrich) was titrated with N-Benzylbenzamide.

Reactions that needed low temperature, were done in refrigerated ketone bath, and to measure the temperature was used a Delta OHM thermometer, model HD 9214. Other reactions were done at room temperature.

Thin layer chromatography (TLC), used to follow the reactions and to check the purity of the compounds, was performed with aluminum plates coated with a silica gel Merck. The revelation of the chromatograms was done with UV irradiation, and using revelators like, p-Anisaldehyde.

All the obtained organic layers were dried using anhydrous Na_2SO_4. The solvents were evaporated in a Buchi rotative rotavapor model 480.

In the purifications done by flash column it was used silica gel (60 Å, ACROS Organics).

To do the HPLC analyses was used a chiral column, Chiracel, and the HPLC used was a Merch HITACHI L-6200 Inteligent Pump, with the detector Merck- Hitachi- L42000 Uv-Vis Detector. The detection was done at 220 nm. The Software used to obtain the data was Clarity - Chromatography SW. Was used as eluents Hexane (CARLO ERBA, ref: 446903) and 2-Propanol (ROML-SpS, ref: H625).

NMR spectra was obtained by a spectrometer Bruker BioSpin GmbH (400 MHz for ^{1}H, and 101 MHz for ^{13}C) belonging to the CMUP. The data was obtained at room temperature and compounds dissolved in deuterated chloroform. Was used as reference the signal of the solvent $CDCl_3$ with a chemical shift of 7.26 ppm (^{1}H) and 77.16 ppm (^{13}C).

The compounds were numbered and named according the IUPAC rules and using the ChemDraw v16.0 program.

The brands of the compounds used are specified below.

Acetyl chloride Merck, cas - 75-36-5, ref: 114189

Benzyldimethylsilane Fluorochem, cas -1631-70-5, ref: S00975

n-BuLi Sigma, cas - 109-72-8, ref: 230707

Dicobalt octacarbonyl Sigma aldrich, cas-10210-68-1, ref: 60811

DIBAL-H Acros, cas - 1191-15-7

DIEA Fluka, cas - 7087-68-5

(+) DIPT Aldrich, cas - 2217-15-4, ref: 229180

DMAP Aldrich, cas - 1122-58-3, ref: 39405

Ethyl Bromoacetate Fluorochem, cas - 105-36-2, ref: BR1168

Sodium acetate cas- 127-09-3

Lypases PS AMANO, AYS AMANO, NOVOZYME 435

NMO Aldrich, cas- 7529-22-8, ref: 224286

Phenylethylamine Fluorochem, cas - 3886-69-9, ref: 03743

PhI(OAc) Fluorochem, cas - 3240-34-4, ref: 091213

PPh_3 Fluorochem – cas - 603-35-0, ref: 037818

PPTS Aldrich, cas - 24057-28-1, ref: 232238

p-TsOH Aldrich, cas - 6192-52-5

TBHP Aldrich, cas - 75-91-2, Ref: 416665

TBTU Bachem, cas - 125700-67-6, Ref: 4013268

$Ti(^iPrO)_4$ Aldrich, cas - 546-68-9

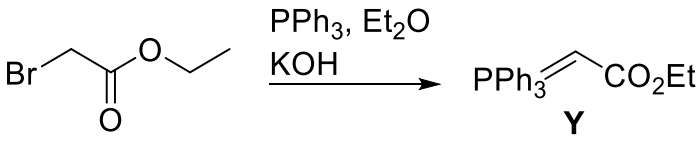
Vinyl acetate Aldrich, 108-05-4

Vinyl magnesium bromide Aldrich, cas - 1826-67-1, Ref: 257257

Z-Ala-OSu BACHEM, cas- 3401-36-3, Ref: 40002

2. Work done in Porto

2.1. Synthesis of Ylide



Y

To a round bottom flask under magnetic stirring PPh_3 (40 g, 153 mmol, 1 eq.) Et_2O (≈250 mL) and ethyl bromoacetate (17 mL, 153 mmol, 1 eq.) were added. This mixture was left to react for 64 hours.

The solution was then cooled in an ice bath and the precipitate was filtered with cooled Et_2O,

The obtained precipitated was dissolved in water (≈500 mL). And then was added a solution of NaOH 1M (50 mL/10 g of salt).

The mixture was transferred to a separation funnel and the organic layer was extracted with DCM (3*250 mL). The organic layer was dried with sodium sulfate, filtered and concentrated in the rotavapor- A yellow oil was obtained.

The ylide was induced to precipitate adding Et_2O. This precipitate was filtered and washed with Et_2O. A white solid was obtained. This compound was then identified by NMR (^1H, ^{13}C, ^{13}C-DEPT). This reaction had a yield of 96%.

Aspect: White solid

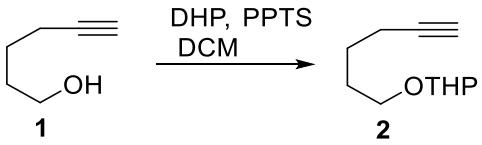
η= 96%

^{1}H NMR (400 MHz, CDCl3) δ 7.98 – 7.11 (15H, m, H_{Ph}), 3.96 (s, 2H, H_{Et}), 2.87 (1H, s, $H_{double\ bond}$), 1.07 (3H, s, H_{Et}).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.17 (C, C=O), 133.20 – 128.66 (C_{Ph}), [60.44 (CH_2), 57.90 (CH_2), C_{Et}], [21.12 (CH), 21.11 (CH), $C_{doublebond}$], [14.88 (CH_3), 14.28 (CH_3), C_{Et}]

2.2. Bicyclic ring 6,5

2.2.1. Synthesis of 2-(hex-5-yn-1-yloxy) tetrahydro-2H pyran (**2**)



In a dry round bottom flask filled with argon was dissolved **1** (4,63 mL, 42,0 mmol, 1 eq) in 45 mL of anhydrous dichloromethane. Then were added to the mixture DHP (5,75 mL, 2,1 mmol, 0,05 eq) and PPTS (5,30, 63,0 mmol, 1,5 eq). This mixture was left stirring for 19 hours at RT. This mixture acquired a yellow color. The reaction was controlled by TLC.

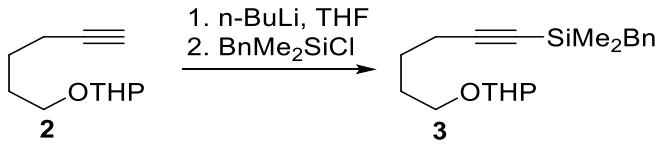
The mixture was transferred to a separation funnel. Then a saturated solution of $NaHCO_3$ was added. The organic layer was extracted with dichloromethane (3 x 20 mL), then washed with water (20 mL) and a saturated solution of NaCl (20 mL). The organic layer obtained was dried with sodium sulfate and concentrated.

After the treatment the concentrated mixture was filtrated through SiO_2, using as eluent a mixture of Et_2O/hexane 40%. Then the mixture was concentrated and putted in vacuum.

Aspect: yellow oil

R_F = 0,80 (20% AcOEt/Hex)

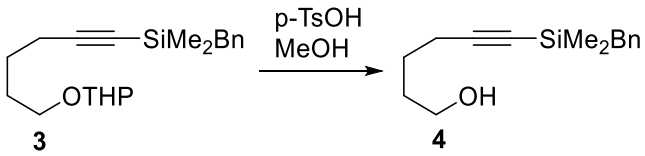
2.2.2. Synthesis of benzyldimethyl(6-((tetrahydro-2H-pyran-2-yl) oxy) hex-1-yn-1-yl) silane (**3**)



In a dry and filled with argon round bottom flask was added **2**, from the previous reaction, and it was dissolved in THF (40 mL). This mixture was kept at -78°C and was slowly added the *n*-BuLi solution (50 mL, 50,1 mmol, 1,1 eq). The mixture was left stirring for 30 minutes. Then $SiMe_2SiCl$ (9,82 mL, 54,6 mmol, 1,3 eq) was added. This mixture was left stirring for 22 hours at room temperature. The reaction was controlled by TLC.

After the solution was cooled to 0°C, 30 mL of a HCl solution of 1 M was added. This mixture was extracted with Et_2O (3 x 10 mL). Then the organic layer was washed with a saturated solution of NaCl. The organic layer was dried with sodium sulfate, filtered and concentrated in the rotavapor. This mixture was directly used in the next step.

2.2.3. Synthesis of 6-(benzyldimethylsilyl)hex-5-yn-1-ol (**4**)



3 **4**

To a round bottom flask containing **3** methanol and a catalytic amount of p-toluenosulphonic acid were added. To stop this reaction 30 mL of a saturated solution of sodium bicarbonate was added and then this mixture was transferred to a separation funnel. The aqueous layer was washed 3 times with 30 mL of ether. The crude was then purified by a medium pressure column of 6*15 cm. First the eluent was hexane, then EtOAc/Hexane 5% and lastly EtOAc/Hexane 10 %. The fractions were collected and then concentrated in the rotavapor. This compound was then identified by NMR (^{1}H, ^{13}C, ^{13}C-DEPT). These three reactions (1.2.1, 1.2.2 and 1.2.3) had a yield of 104 %.

Aspect: yellow oil

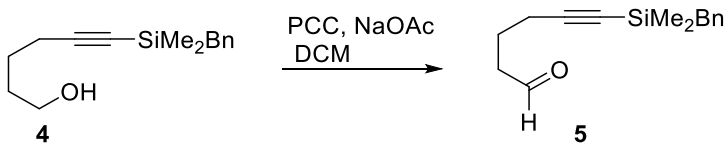
Ƞ = 104% (existence of solvent)

R_F = 0,38 (20% AcOEt/Hex)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 6.86 (5H, m, H_{Ph}), 3.59 (2H, t, *J* = 6.2 Hz, H-1), 2.22 (2H, t, *J* = 6.8 Hz, H-4), 2.15 (2H, s, CH_2Si), 1.98 (1H, s, OH), 1.68 – 1.47 (4H, m, C-3 and C-4), 0.08 (6H, s, Si-Me_2)

^{13}C NMR (101 MHz, $CDCl_3$) δ 139.27 (C, C_{Ph}), 128.40 (2*CH, C_{Ph}), 128.13 (2*CH, C_{Ph}), 124.28 (CH, C_{Ph}), 108.65 (C, C-6), 83.26 (C, C-5), 62.20 (CH_2, C-1), 31.73 (CH_2), 26.52 (CH_2), 24.84 (CH_2), 19.66 (CH_2), -1.85 (2*CH_3, 2CH_3-Si).

2.2.4. Synthesis of 6-(benzyldimethylsilyl) hex-5-ynal (**5**)



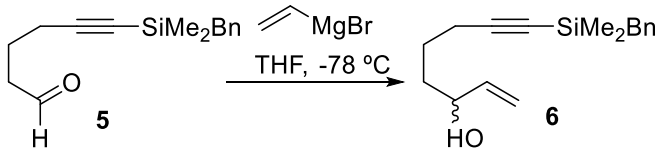
In a 500 mL flask DCM (200 mL), PCC (18,9 g, 88,0 mmol, 2 eq), NaOAc (4,15 g, 50,6 mmol, 1.15 eq), and molecular filters (5,03 g) were added. Then the compound **4** was added to the mixture and this mixture was left stirring for 2,5 hours at the room temperature.

The crude obtained was filtered twice by SiO_2 and washed with Et_2O. The compound was dried in vacuum.

Aspect: Yellow oil

R_F = 0,69 (20% AcOEt/Hex)

2.2.5. Synthesis of 8-(benzyldimethylsilyl)oct-1-en-7-yn-3-ol (**6**)



To the crude obtained before, compound **5**, THF (80 mL) and a solution of Vinylmagnesium bromide in THF (66 mL, 66,0 mmol, 1,5 eq.) were added. This mixture was left stirring for 15 hours and was controlled by TLC. Then the reaction mixture was transferred to a separation funnel and a saturated solution of NH_4Cl (80 mL) and a saturated solution of NaCl (80 Ml) were added. The organic layer was extracted with Et_2O (3*50 mL) and then washed with a saturated solution of NaCl (50 mL). Then was dried with sodium sulfate, filtered and concentrated in the rotavapor.

The obtained crude was purified using a SiO_2 column (8 x 6 cm). As eluents were used firstly hexane, then 10% of EtOAc/ Hexane and for the last fractions 20% of EtOAc/Hexane.The fractions were collected and concentrated in the rotavapor. This compound was then identified by NMR (^1H, ^{13}C, ^{13}C-DEPT). This reaction had a yield of 67%.

Aspect: Yellow oil

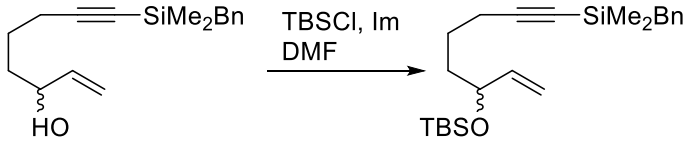
η = 67 %

R_F = 0,57 (20% AcOEt/Hex)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 – 7.04 (5H, m, H_{Ph}), 5.93 – 5.82 (1H, m, H-2), 5.24 (1H, dt, *J* = 17.2, 1.4 Hz, H-1), 5.13 (1H, dt, *J* = 10.4, 1.3 Hz, H-1), 4.14 (1H, q, *J* = 6.0 Hz, H-3), 2.27 (2H, t, *J* = 6.8 Hz, H-6), 2.18 (2H, s, CH_2Si), 1.69 – 1.58 (4H, m, H-4 and H-5), 1.56 (1H, s, OH), 0.11 (6H,s, Si-Me_2).

^{13}C NMR (101 MHz, $CDCl_3$) δ 141.14 (CH, C-2), 139.39 (C,C_{Ph}), 128.51 (2*CH, C_{Ph}), 128.24 (2*CH,C_{Ph}), 124.38 (CH, C_{Ph}), 114.97 (CH_2, C-1), 108.63 (C, C-8), 83.43 (C, C-7), 72.85 (CH, C-3), 36.10 (CH_2), 26.64 (CH_2), 24.45 (CH_2), 19.89 (CH_2), -1.77 (2*CH_3, 2CH_3-Si).

2.2.6. Synthesis of benzyl(6-((tert-butyldimethylsilyl) oxy)oct-7-en-1-yn-1-yl)dimethylsilane (**7**)



6 **7**

In a dry and filled with argon round bottom flask, with the compound **6**, DMF (200 mL) and Imidazol (6,79 g, 100 mmol, 3,3 eq.) were added. This mixture was left stirring for 10 minutes.

To the obtained mixture was added TBSCl (12,39g, 82 mmol, 2,7 eq.). This mixture was left stirring until no starting material was observed (4 days). The obtained mixture was transferred to a separation funnel and a saturated solution of NaCl (40 mL) and a saturated solution of NH_4Cl (40 mL) were added. The organic layer was extracted using Et_2O., then dried with sodium sulfate, filtered and concentrated in the rotavapor.

The obtained crude was purified by column of SiO_2 (8 x 6 cm). As eluent were used initially only hexane and lastly 10 % EtOAc/ Hexane. The compound was then concentrated in the rotavapor and identified by NMR (^{1}H, ^{13}C and ^{13}C-DEPT). This reaction had a yield of 84%.

Aspect: transparent oil

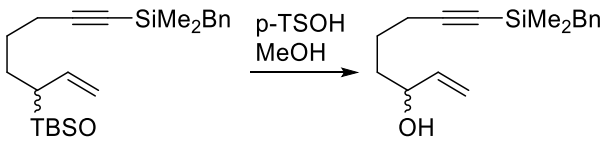
η = 84%

R_F = 0,94 (20% AcOEt/Hex)

^{1}H NMR (400 MHz, CDCl3) δ 7.25 – 7.03 (5H, m, H_{Ph}), 5.86 – 5.75 (1H, m, H-7), 5.20 – 5.14 (1H, m, H-8), 5.08 – 5.03 (1H, m, H-8), 4.14 (1H, q, *J* = 5.7 Hz, H-6), 2.24 (2H, t, J = 6.5 Hz, H-3), 2.18 (2H, s, CH_2Si), 1.69 – 1.50 (4H,m, H-4 and H-5), 0.92 (9H,s, *t*-Bu), 0.10 (6H, s, Si-Me_2), 0.07 (3H, s, CH_3-Si), 0.05 (3H, s, CH_3-Si).

^{13}C NMR (101 MHz, $CDCl_3$) δ 141.64 (CH, C-7), 139.43 (C, C_{Ph}), 128.52 (2*CH, C_{Ph}), 128.25 (2*CH, C_{Ph}), 124.37 (CH, C_{Ph}), 113.93 (CH_2, C-8), 108.92 (C, C-1), 83.16 (C, C-2), 73.49 (CH, C-6), 37.13 (CH_2), 26.69 (CH_2), 26.05 (3*CH_3, *t*-Bu), 24.25 (CH_2), 20.02 (CH_2), 18.41 (C, C-Si), -1.77 (2*CH_3, Si-Me_2), -4.23 (CH_3, CH_3-Si), -4.66 (CH_3, CH_3-Si).

2.2.7. Synthesis of 8-(benzyldimethylsilyl)oct-1-en-7-yn-3-ol (**6**)



7 **6**

To a 250 mL round bottom flask compound **7** (0,825g, 2,13 mmol, 1 eq.), MeOH (100 mL) and p-TSOH (0,02 g, 0,11 mmol, 0,05 eq.) were added.

The mixture was concentrated in the rotavapor and transferred to a separation funnel. To the separation funnel a saturated solution of $NaCO_3$ (50 mL) was added. The organic layer was extracted using Et_2O, dried with sodium sulfate and concentrated in the rotavapor. The compound obtained was pure so wasn't done a purification.

The compound was then concentrated in the rotavapor and identified by NMR (^{1}H, ^{13}C and ^{13}C-DEPT).

Aspect: transparent oil

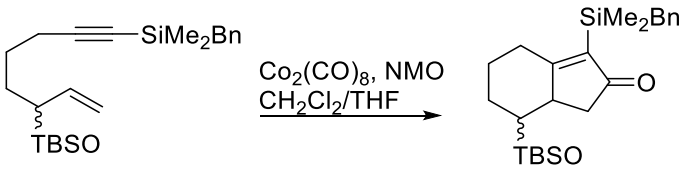
η = >100% (solvent)

R_F = 0,43 (20% EtOAc/Hexane)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 – 7.04 (5H, m, H_{Ph}), 5.93 – 5.82 (1H, m, H-2), 5.24 (1H, dt, *J* = 17.2, 1.4 Hz, H-1), 5.13 (1H, dt, *J* = 10.4, 1.3 Hz, H-1), 4.14 (1H, q, *J* = 6.0 Hz, H-3), 2.27 (2H, t, *J* = 6.8 Hz, H-6), 2.18 (2H, s, CH_2Si), 1.69 – 1.58 (4H, m, H-4 and H-5), 1.56 (1H, s, OH), 0.11 (6H,s, Si-Me_2).

^{13}C NMR (101 MHz, $CDCl_3$) δ 141.14 (CH, C-2), 139.39 (C,C_{Ph}), 128.51 (2*CH, C_{Ph}), 128.24 (2*CH,C_{Ph}), 124.38 (CH, C_{Ph}), 114.97 (CH_2, C-1), 108.63 (C, C-8), 83.43 (C, C-7), 72.85 (CH, C-3), 36.10 (CH_2), 26.64 (CH_2), 24.45 (CH_2), 19.89 (CH_2), -1.77 (2*CH_3, 2CH_3-Si).

2.2.8. Synthesis of 3-(benzyldimethylsilyl)-7-((tert-butyldimethylsilyl)oxy)-1,4,5,6,7,7a-hexahydro-2H-inden-2-one (**9**)



7 **9**

To a dry and filled with argon round bottom flask, was transferred **7** (2,117g, 5,62mmol, 1 eq.) and DCM (100 mL) was added. To the mixture $Co_2(CO)_8$ (2,5g, 7,31 mmol, 1,3 eq.) was added and it acquired a brown color. This mixture was left stirring for 2 hours, protected from the light.

Then DCM (120 mL) and THF (180 mL) were added to the solution. This solution was cooled in an ice bath. Then was added to the mixture NMO (7,90 g, 6,74 mmol, 1,2 eq). This reaction was left stirring for 20 hours.

The reaction mixture was filtered through SiO_2 and washed with 20% EtOAc/Hexane and concentrated in the rotavapor.

To purify the compound a flash chromatography (4*7 cm) was done using as eluent 1% EtOAc/Hexane. The compound was then concentrated in the rotavapor and identified by NMR (^1H, ^{13}C and ^{13}C-DEPT).

Aspect: yellow oill

ɳ = 67%

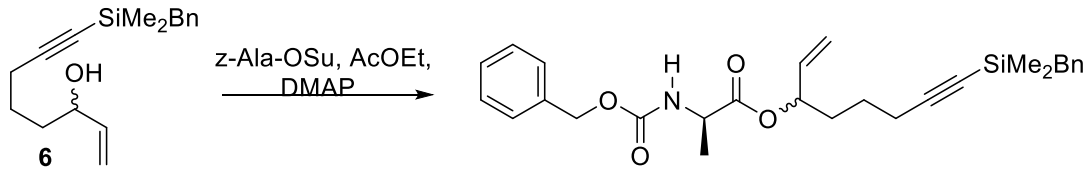
R_F = 0,48 (5% EtOAc/Hexane)

^1H NMR (400 MHz, $CDCl_3$) δ 7.21 – 6.85 (5H, m,H_{Ph}), 3.19 – 3.07 (1H, m, H-7), 2.70 – 2.47 (3H, m), 2.34 (1H,d, *J* = 13.5 Hz, CH_2Si), 2.25 (1H, d, *J* = 13.4 Hz, CH_2Si), 2.15 (1H, d, *J* = 16.9 Hz, H-7a), 1.88 (2H, m, H-1), 1.36 (3H,m), 0.90 (9H,s, *t*-Bu), 0.23 (6H, s, Si-Me_2), 0.07 (3H,s, CH_3-Si), 0.06 (3H, s, CH_3-Si

^{13}C NMR (101 MHz, $CDCl_3$) δ 213.1 (C, C-2), 189.4 (C, C-3a), 140.3 (C, C-3), 136.4 (C), 128.38 (2*CH_2, C_{Ph}) 128.15 (2*CH_2, C_{Ph}), 124.10 (C, C_{Ph}), 77.76 (CH, C-7), 52.88(CH, C-7a) 41.00 (CH_2), 35.16(CH_2), 30.5(CH_2), 25.90 (3*CH_3, *t*-Bu), 25.52 (CH_2), 23.82 (CH_2),

18.09 (C, *t*-Bu), -2.01 (CH3, CH_3-Si), -2.17 (CH3, CH_3-Si), -3.82 (CH3, CH_3-Si), -4.48 (CH3, CH_3-Si)

2.2.9. Resolution attempt 1



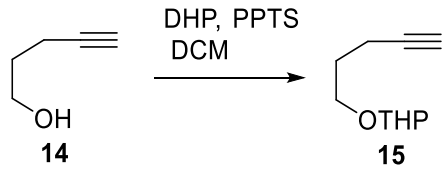
To a 25 mL round bottom flask **6** (0,07 g, 0,3 mmol, 1 eq), EtOAc (10 mL) and z-Ala-OSu (0,08 g, 0,3 mmol, 1 eq) were added. Then was added a catalytic quantity of DMAP. The crude was then concentrated and purified by chromatographic SiO_2 column (8*2 cm). As eluent was used 25 % EtOAc/Hexane.

Aspect: yellow oil

R_F = 0,42 (20 % EtOAc/Hex)

2.3. Bicyclic ring 5,5

2.3.1. Synthesis of 2-(pent-4-yn-1-yloxy)tetrahydro-2H-pyran (**15**)



To a 100 mL reaction tube dried and filled with argon, compound **14** (7,94 mL, 84 mmol, 1 eq) was added. Then PPTS (1,06 g, 4,2 mmol, 0,05 eq.), DHP (11,50 mL, 126 mmol, 1,5 eq.) and dried DCM (100 mL) were added. This mixture was left stirring for 17 hours.

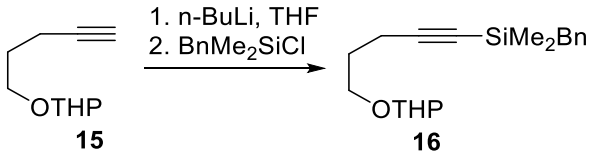
Then the mixture was transferred to a separation funnel and a saturated solution of $NAHCO_3$ (40 mL) was added. The organic layer was extracted using DCM (3 *16 mL) and washed with water and a saturated solution of NaCl (20 mL). Then was dried, filtrated and concentrated in the rotavapor.

The crude was purified by SiO_2 column and as eluent Et_2O/hexane was used. This was then filtered and concentrated in the rotavapor.

Aspect: yellow oil

R_f = 0,70 (20% AcOEt/Hex)

2.3.2. Synthesis of benzyldimethyl(5-((tetrahydro-2H-pyran-2-yl) oxy)pent-1-yn-1-yl) silane (**16**)

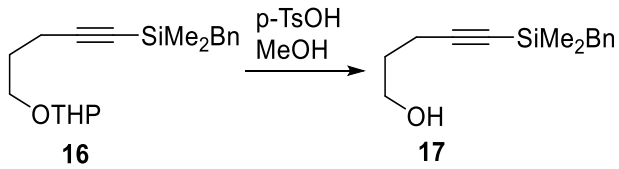


To the compound **15** THF (80 mL) was added. This mixture was cooled and a solution of n-BuLi in hexane (100 mL, 101 mmol, 1,2 eq.) was added. This mixture was left stirring for 30 minutes. To the reaction mixture $SiBuMe_2Cl$ (17,09 mL, 109 mmol, 1,3 eq.) was added and was left stirring for 22 hours. The mixture was cooled at 0°C and an aqueous solution of HCl (60 mL, 1M) was added. The mixture was transferred to a separation funnel and the organic layer was extracted with Et_2O (3*20 mL) and washed with a saturated solution of NaCl. Then the organic layer was dried with sodium sulfate, filtered and concentrated in the rotavapor and used directly in the next step.

Aspect: yellow oil

R_F = 0,94 (20% AcOEt/Hex)

2.3.3. Synthesis of 5-(benzyldimethylsilyl)pent-4-yn-1-ol (**17**)



To the obtained mixture 80 mL of MeOH, and p-TsOH (1,20 g, 6,3 mmol, 0,075 eq.) were added. The mixture was left stirring for about two and a half hours.

To stop the reaction Et_3N (≈ 0,88 mL, 0,075eq.) was added. The mixture was concentrated in the rotavapor, and then transferred to a separation funnel. The organic layer was washed with a saturated solution of NaCL (1*30 mL) and then extracted with CH_2Cl_2 (3*20 mL). The organic layer was then dried with sodium sulfate, filtered and concentrated in the rotavapor.

The crude was purified by a column of SiO_2 column (10,5*5 cm). To elute the pretended compound first was used only hexane, and then the polarity was gradually increased with

the use of Ethyl acetate, by 5% Ethyl acetate in hexane, and then 10% ethyl acetate in Hexane.

Then was made another column in which was used the same eluents as above, hexane, for the first fraction, then 5% ethyl acetate in hexane and lastly 10% ethyl acetate in hexane.

This product was then identified by RMN (^{1}H, ^{13}C and ^{13}C-DEPT). The yield was 85%, for the three steps (1.3.1, 1.3.2 and 1.3.3). Was obtained a yellow oil.

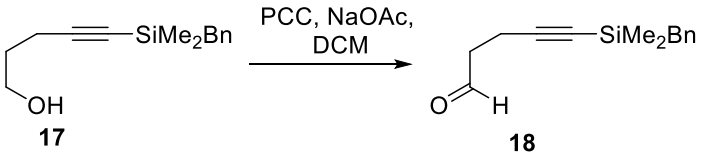
Aspect: yellow oil

η = 85%

R_f = 0,37 (20% AcOEt/Hex)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 – 7.04 (5H, m, H_{Ph}), 3.71 (2H, t, *J* = 6.1 Hz, H-1), 2.34 (2H, t, *J* = 6.9 Hz, H-3), 2.17 (2H ,s, CH_2Si), 1.81 – 1.66 (3H, m, H-2 and OH), 0.11 (6H, s, Si-Me_2)

2.3.4. Synthesis of 5-(benzyldimethylsilyl)pent-4-ynal (**18**)



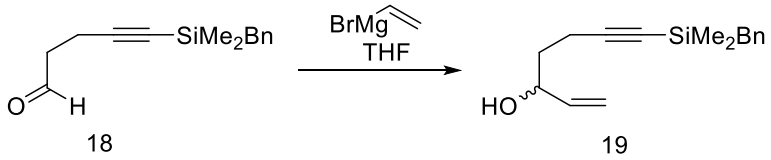
In a dried and protected with argon reactor dried DCM (≈400 mL), NaOAc (6,74g, 82,1 mmol, 1,15 eq.) and the PCC (30,94g, 143 mmol, 2 eq.) were added, then carefully the compound **17** was added**.** This mixture was left stirring for two and a half hours. In this procedure we used 12 grams of SiO_2 instead of the molecular sieves. This reaction, initially orange, changed the color to a very dark brown in the end of the reaction.

The mixture was filtered through SiO_2 to a bottom round flask, washed with Et_2O and concentrated. Then the mixture was transferred, under argon, to a dried Schlenk flask and was concentrated.

Aspect: yellow oil

R_f = 0,69 (20% AcOEt/Hex)

2.3.5. Synthesis of 7-(benzyldimethylsilyl)hept-1-en-6-yn-3-ol (**19**)



18 19

To a protected with argon Schlenk flask, containing the mixture from the previous reaction THF (≈160 mL) was added and using a cannula a solution of Vinylmagnesium bromide in THF (≈120 mL, 107mmol, 1,5 eq.) was added at -78 °C. The mixture was left stirring at room temperature for 16 hours. Then to the reaction a saturated solution of NH_4Cl (30 mL) and NaCl (30 mL) were added. The organic layer was extracted with Et_2O (3*30 mL) and then dried with sodium sulfate, filtered and concentrated in the rotavapor.

The crude was purified through a SiO_2 column (6*8 cm) and was used as a solvent firstly only hexane and then 5 % EtOAc/Hex. The obtained yield was 71%.

Aspect: yellow oil

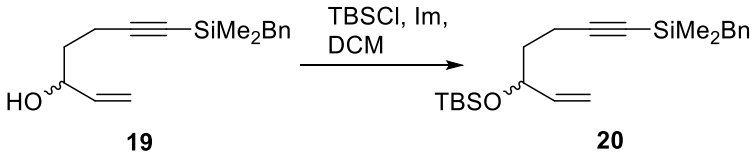
ɳ = 71%

R_f = 0,54 (20% AcOEt/Hex)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (5H, m, H_{Ph}), 5.94 – 5.78 (1H, m, H-2), 5.27 (1H, dt, *J* = 17.6, 1.4 Hz, H-1), 5.15 (1H, dt, *J* = 10.4, 1.3 Hz, H-1), 4.24 (1H, dd, *J* = 12.7, 6.0 Hz, H-3), 2.44 – 2.28 (2H, m, H-5), 2.19 (2H, s, CH_2Si), 2.02 (1H ,s, OH), 1.73 (2H, dd, *J* = 14.1, 6.9 Hz, H-4), 0.13 (6H, s, Si-Me_2)

^{13}C NMR (101 MHz, $CDCl_3$) δ 140.47 (CH, C-2), 139.29 (C, C_{Ph}), 128.47 (2*CH, C_{Ph}), 128.23 (2*CH, C_{Ph}), 124.39 (CH, C_{Ph}), 115.17 (CH_2, C-1), 108.24 (C, C-7), 83.81 (C, C-6), 72.00 (CH, C-3), 35.46 (CH_2), 26.55 (CH_2), 16.25 (CH_2), -1.81 (2*CH_3, 2CH_3-Si).

2.3.6. Synthesis of benzyl(5-((tert-butyldimethylsilyl) oxy)hept-6-en-1-yn-1-yl)dimethylsilane (**20**)



19 **20**

To the obtained mixture in the previous reaction imidazole (15,64 g, 230mmol, 4,50 eq.) and dried DCM (≈350 mL) were added. The mixture was left stirring for 10 minutes. After the 10 minutes TBSCl (15,96 g, 106 mmol, 2 eq.) was added. This reaction was controlled by TLC. The mixture was transferred to a separation funnel and saturated solutions of NaCl (30 mL) and NH_4Cl (30 mL) were added. Then the organic layer was extracted with DCM (4*30 mL), was dried with sodium sulfate, filtered and concentrated in the rotavapor.

The crude was purified using a chromatographic column with SIO_2 (6*8 cm). As eluent was firstly used hexane, then 5% EtOAc/Hex and lastly 10% EtOAc/Hex. This reaction had a yield of 64 %. This product was then identified by NMR (^1H, ^{13}C and ^{13}C-DEPT). A yellow oil was obtained.

Aspect: yellow oil

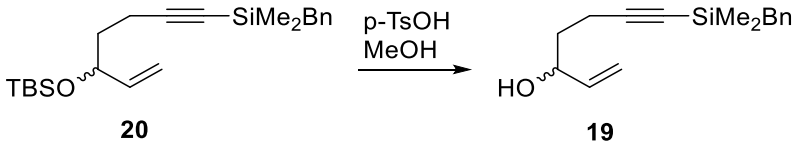
ɳ = 64%.

R_f = 0,91 (20% AcOEt/Hex)

^1H NMR (400 MHz, $CDCl_3$) δ 7.16 (5H,m, H_{Ph}), 5.86 – 5.74 (1H, m, H-6), 5.13 (2H, m, H-7), 4.23 (1H, dd, *J* = 12.3, 6.2 Hz, H-5), 2.29 (2H, dd, *J* = 14.7, 7.4 Hz, H-3), 2.19 (2H , s, CH_2Si), 1.77 – 1.63 (2H, m, H-4), 0.91 (9H, s, *t*-Bu), 0.11 (6H, s, Si-Me_2), 0.08 (3H, s, CH_3-Si), 0.05 (3H, s, CH_3-Si)

^{13}C NMR (101 MHz, $CDCl_3$) δ 141.24 (CH, C-6), 139.40 (C, C_{Ph}), 128.51 (2*CH, C_{Ph}), 128.27 (2*CH, C_{Ph}), 124.41 (CH, C_{Ph}), 114.37 (CH_2, C-7), 108.79 (C, C-1), 83.31 (C, C-2), 72.45 (CH, C-5), 36.81 (CH_2), 26.66 (CH_2), 26.05 (3*CH_3, *t*-Bu), 18.38 (C, C-Si), 16.02 (CH_2), -1.77 (2*CH_3, Si-Me_2), -4.20 (CH_3, CH_3-Si), -4.72 (CH_3, CH_3-Si).

2.3.7. Synthesis of 7-(benzyldimethylsilyl)hept-1-en-6-yn-3-ol (**19**)



20 **19**

To a 250 mL round bottom flask compound **19** (11,25g, 30,18 mmol, 1 eq.) was added. To the flask MeOH (250 mL) and p-TsOH (0,26 g, 0,11 mmol, 0,05 eq.) were added. The reaction was left to react for one day.

The mixture was concentrated in the rotavapor and transferred to a separation funnel. To the separation funnel a saturated solution of $NaCO_3$ (100 mL) was added. The organic layer was extracted using Et_2O, then dried with sodium sulfate and concentrated in the rotavapor. The compound was purified by a chromatography column of SiO_2 (4*15 cm) and as eluent was used firstly 5%EtOAc/Hex, 10%EtOAc/Hex, 20%EtOAc/Hex. The compound was then concentrated in the rotavapor and identified by NMR (^{1}H, ^{13}C and ^{13}C-DEPT). This reaction had a yield of 89%.

Aspect: yellow oil

ɳ = 89%

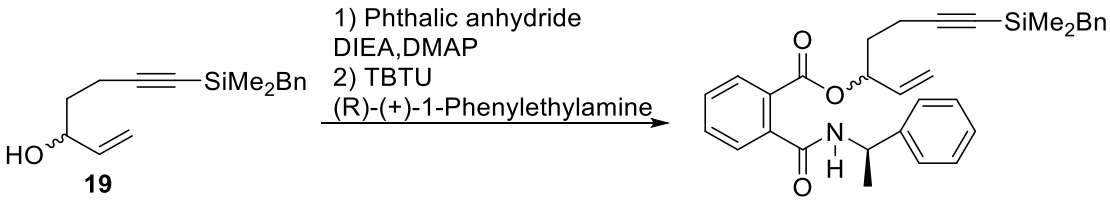
R_F = 0,43 (20% AcOEt/Hex)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (5H, m, H_{Ph}), 5.94 – 5.78 (1H, m, H-2), 5.27 (1H, dt, *J* = 17.6, 1.4 Hz, H-1), 5.15 (1H, dt, *J* = 10.4, 1.3 Hz, H-1), 4.24 (1H, dd, *J* = 12.7, 6.0 Hz, H-3), 2.44 – 2.28 (2H, m, H-5), 2.19 (2H, s, CH_2Si), 2.02 (1H ,s, OH), 1.73 (2H, dd, *J* = 14.1, 6.9 Hz, H-4), 0.13 (6H, s, Si-Me_2)

^{13}C NMR (101 MHz, $CDCl_3$) δ 140.47 (CH, C-2), 139.29 (C, C_{Ph}), 128.47 (2*CH, C_{Ph}), 128.23 (2*CH, C_{Ph}), 124.39 (CH, C_{Ph}), 115.17 (CH_2, C-1), 108.24 (C, C-7), 83.81 (C, C-6), 72.00 (CH, C-3), 35.46 (CH_2), 26.55 (CH_2), 16.25 (CH_2), -1.81 (2*CH_3, 2CH_3-Si).

.

2.3.8. Resolution attempt 2

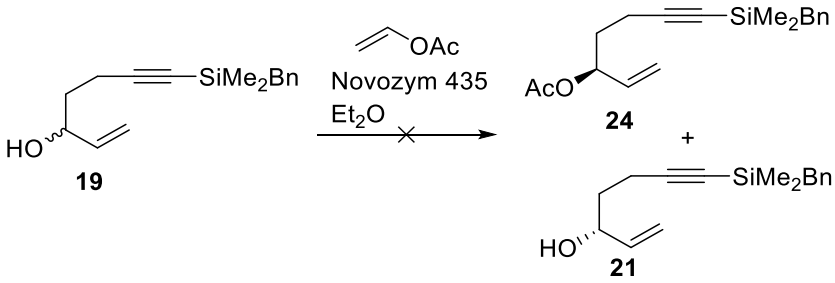


In a 100 mL round bottom flask 50 mL of DCM and (0,53g, 2,04mmol, 1 eq.) of the compound **19** were added. Then the two bases DIEA (0,28g, 2,24 mmol, 1.1 eq.) and DMAP (0,27g, 2,24 mmol, 1,1 eq.) were added. After the mixture was very well dissolved phthalic anhydride (0,60 g, 4,08 mmol, 2 eq.) was added. This reaction was left to react for one week (the time needed for the starting material almost disappear). Then TBTU (0,66 g, 2,04 mmol, 1 eq.) was added. This mixture was left for 15 minutes, the time needed to the TBTU became dissolved in the mixture. Finally, (R)-Phenylethylamine (0,650mL, 5,1 mmol, 2,5 eq.) was added

Aspect: yellow oil

R_F = 0,40 (dichloromethane)

2.3.9. Resolution attempt 3



To a round bottom flask **19** (0,52 g, 2 mmol, 1 eq), vinyl acetate (0,090 mL, 0,96 mmol, 0,49 eq), enzyme Novozyme 435 (0,26 g) and Et_2O (15 mL) were added. This reaction was left reacting in orbital stirring for 32 hours.

The mixture was filtrated using Et_2O and then concentrated. The obtained crude was purified using a SiO_2 column (8*15 cm), using dichloromethane as eluent. The compound was concentrated and identified by NMR (^1H, ^{13}C and ^{13}C-DEPT).

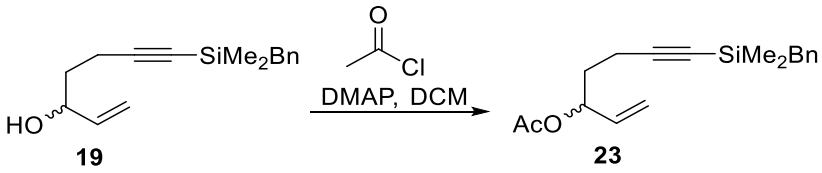
Aspect: yellow oil

R_F = 0,90-acetilated and 0,50-alchool (dichloromethane)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 – 7.02 (5H, m, H_{Ph}), 5.78 (1H, m, H-2), 5.36 – 5.30 (1H, m, H-3), 5.27 (1H, dt, *J* = 17.3, 1.3 Hz, H-1), 5.21 (1H, dt, *J* = 10.5, 1.2 Hz, H-1), 2.28 (2H, t, *J* = 7.4 Hz, H-5), 2.19 (2H, s, CH_2Si), 2.07 (3H, s, H_{AcO}), 1.86 (2H, m, H-4), 0.11 (6H, s, Si-Me_2).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.17 (C, C=O), 139.27 (C, C_{Ph}), 135.84 (CH, C-2), 128.47 (2*CH, C_{Ph}), 128.22 (2*CH, C_{Ph}), 124.37 (CH, C_{Ph}), 117.27 (CH_2, C-1), 107.36 (C, C-7), 83.79 (C, C-6), 73.64 (CH, C-3), 33.09 (CH_2), 26.52 (CH_2), 21.24 (CH_3,AcO), 16.02 (CH_2), -1.87 (2*CH_3, 2CH_3-Si).

2.3.10. Synthesis of 7-(benzyldimethylsilyl)hept-1-en-6-yn-3-yl acetate (**23**)



To a 25 mL round bottom flask **19** (0,50 g, 1,9 mmol, 1 eq), DCM (12mL) and DMAP (0,27 g, 2,24 mmol, 1,1 eq) were added. In the end the acetyl chloride was added. This reaction was left string and controlled by TLC.

Then the mixture was purified using an SiO_2 column (25*4 cm), and as eluent dichloromethane was used. The compound was concentrated and identified by NMR (^{1}H, ^{13}C and ^{13}C-DEPT).

Aspect: yellow oil

Ƞ = 89%

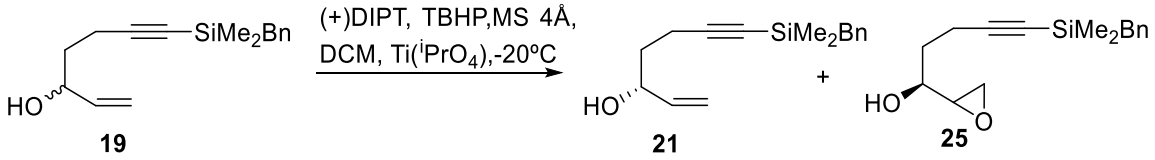
R_F = 0,90 (dichloromethane)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 – 7.02 (5H, m, H_{Ph}), 5.78 (1H, m, H-2), 5.36 – 5.30 (1H, m, H-3), 5.27 (1H, dt, *J* = 17.3, 1.3 Hz, H-1), 5.21 (1H, dt, *J* = 10.5, 1.2 Hz, H-1), 2.28 (2H, t, *J* = 7.4 Hz, H-5), 2.19 (2H, s, CH_2Si), 2.07 (3H, s, H_{AcO}), 1.86 (2H, m, H-4), 0.11 (6H, s, Si-Me_2).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.17 (C, C=O), 139.27 (C, C_{Ph}), 135.84 (CH, C-2), 128.47 (2*CH, C_{Ph}), 128.22 (2*CH, C_{Ph}), 124.37 (CH, C_{Ph}), 117.27 (CH_2, C-1), 107.36 (C, C-7),

83.79 (C, C-6), 73.64 (CH, C-3), 33.09 (CH_2), 26.52 (CH_2), 21.24 (CH_3,AcO), 16.02 (CH_2), -1.87 (2*CH_3, 2CH_3-Si).

2.3.11. Resolution to obtain the alcohol (**21**)



To a protected with argon Schlenk flask, with molecular sieves compound **19** (1,57 g, 6,1 mmol, 1eq.), dichloromethane (20 mL) and (+) DIPT (1,80 mL, 7,3 mmol, 1,2 eq) were added. This mixture was left stirring at -18,5°C for 30 minutes. Then Ti($^{i}PrO_4$) (1,50 mL, 6,1 mmol, 1 eq.) was added and was left string for more 30 minutes. Then TBHP in decane (0,80 mL, 5,5 M, 0,7 eq) was added. This mixture was left at -20 °C for 21 days.

The reaction was stopped using a mixture of $FeSO_4.7H_2O$ (3,50 g), tartaric acid (1,14 g) and H_2O (20 mL). The organic layer was extracted with Et_2O (3*20 mL). The organic layers were combined, dried with sodium sulfate, filtered and then concentrated in the rotavapor. Then the crude was purified through flash chromatography (SiO_2, 8*15 cm) using as eluent 10 % EtOAc/Hexane. The compound was concentrated and identified by NMR (^{1}H, ^{13}C and ^{13}C-DEPT).

Aspect: yellow oil

R_F = 0,70-alcohol (10% EtOAc/Hex)

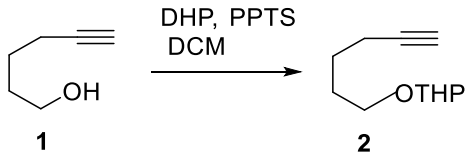
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (5H, m, H_{Ph}), 5.94 – 5.78 (1H, m, H-2), 5.27 (1H, dt, *J* = 17.6, 1.4 Hz, H-1), 5.15 (1H, dt, *J* = 10.4, 1.3 Hz, H-1), 4.24 (1H, dd, *J* = 12.7, 6.0 Hz, H-3), 2.44 – 2.28 (2H, m, H-5), 2.19 (2H, s, CH_2Si), 2.02 (1H ,s, OH), 1.73 (2H, dd, *J* = 14.1, 6.9 Hz, H-4), 0.13 (6H, s, Si-Me_2)

^{13}C NMR (101 MHz, $CDCl_3$) δ 140.47 (CH, C-2), 139.29 (C, C_{Ph}), 128.47 (2*CH, C_{Ph}), 128.23 (2*CH, C_{Ph}), 124.39 (CH, C_{Ph}), 115.17 (CH_2, C-1), 108.24 (C, C-7), 83.81 (C, C-6), 72.00 (CH, C-3), 35.46 (CH_2), 26.55 (CH_2), 16.25 (CH_2), -1.81 (2*CH_3, 2CH_3-Si).

3. Work done in Spain

3.1. Bicyclic ring 6,5

3.1.1. Synthesis of 2-(hex-5-yn-1-yloxy) tetrahydro-2H pyran (**2**)



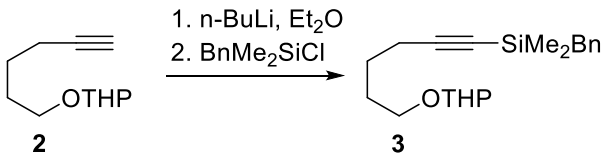
To a 250 mL round bottom flask compound **1** (15 g, 152 mmol, 1 eq.), dried DCM (≈100 mL) and PPTS (1,9 g, 7,64 mmol, 0,05 eq.) were added. This mixture was left to react for 3 days. Was formed a clean solution. The mixture then was concentrated in the rotavapor and purified using a SiO_2 column (5*10 cm). As eluent was used 10% EtOAc/Hex.

This same experience was repeated. In the total were used 30 grams of the compound **1**.

Aspect: Yellow oil

R_F = 0,80 (20% AcOEt/Hex)

3.1.2. Synthesis of Synthesis of benzyldimethyl(6-((tetrahydro-2H-pyran-2-yl) oxy) hex-1-yn-1-yl) silane (**3**)



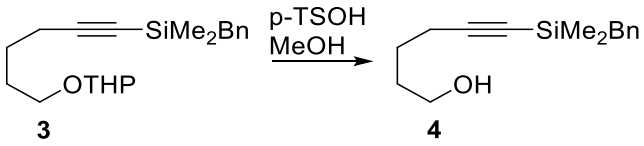
Using a cannula compound **2** (32,7 g, 179 mmol, 1 eq.) was transferred to a 500 mL round bottom flask, already containing Et_2O (200 mL). The mixture was cooled and then was slowly added the n-BuLi (79 mL, 197 mmol, 1,1 eq.). This reaction was left stirring 1 hour out of the cold bath. The mixture was cooled again at 50 °C and the $ClSiMe_2Bn$ was added. Then the mixture was taken from the bath and left stirring for 24 hours.

To stop the reaction some water drops were added. Then the mixture was transferred to a separation funnel and was washed with H_2O (100 mL) and the organic layer was extracted with Et_2O (150 mL). The organic layer was dried with sodium sulfate, filtered and then concentrated in the rotavapor.

To purify the crude a chromatography column of SiO_2, (5*14cm) was used and as eluent hexane, 5% EtOAc/Hex and lastly 10 % EtOAc/Hex were used.

This reaction was done again but using 29,45 grams of **2** compound and using as solvent THF.

3.1.3. Synthesis of Synthesis of 6-(benzyldimethylsilyl) hex-5-yn-1-ol (**4**)



3 **4**

To a round bottom flask containing **3** (60 g, 181 mmol, 1 eq.) p-TSOH (1,8g; 9,46 mmol, 0,05eq.) was added. The mixture was left to react for 24 hours, protected from light. The mixture was concentrated in the rotavapor and then a saturated solution of sodium bicarbonate (5 mL) was added. The mixture was then transferred to a separation funnel, and the organic layer was washed with water and extracted with Et_2O.

To purify the crude was necessary to do two chromatography columns of SiO_2 (7*20 cm) and as eluent 5% EtOAc/Hex and 10%EtOAc/Hex were used.

This reaction was done again but using 53 grams of the compound **3.**

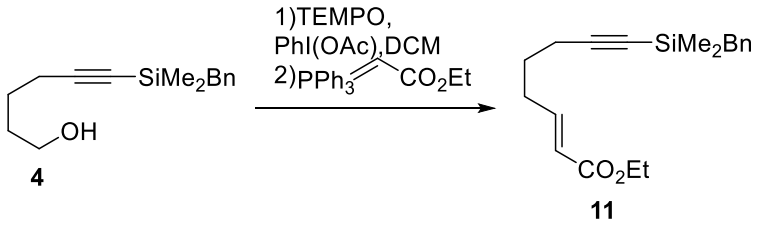
Aspect: Yellow oil

R_F = 0,38(20% AcOEt/Hex)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 6.86 (5H, m, H_{Ph}), 3.59 (2H, t, *J* = 6.2 Hz, H-1), 2.22 (2H, t, *J* = 6.8 Hz, H-4), 2.15 (2H, s, CH_2Si), 1.98 (1H, s, OH), 1.68 – 1.47 (4H, m, C-3 and C-4), 0.08 (6H, s, Si-Me_2)

^{13}C NMR (101 MHz, $CDCl_3$) δ 139.27 (C, C_{Ph}), 128.40 (2*CH, C_{Ph}), 128.13 (2*CH, C_{Ph}), 124.28 (CH, C_{Ph}), 108.65 (C, C-6), 83.26 (C, C-5), 62.20 (CH_2, C-1), 31.73 (CH_2), 26.52 (CH_2), 24.84 (CH_2), 19.66 (CH_2), -1.85 (2*CH_3, 2CH_3-Si).

3.1.4. Synthesis of Ethyl (E)-8-(benzyldimethylsilyl)oct-2-en-7-ynoate (**11**)



In a round bottom flask containing the compound **4** (18,9g, 76,7 mmol, 1eq) dried DCM (300mL) was added. Then to the solution $PhI(OAc)_2$ (22,72g, 70,54mmol, 0,9 eq.) and TEMPO (1,7g, 10 mmol, 0,13 eq.) were added. This mixture was left stirring protected from the light for 5 hours. Then to the mixture ylide (24,6 g; 70,54;0,9 eq.) was added.

The mixture was the transferred to a separation funnel and washed with a saturated solution of Na_2SO_4 (2*150mL). The organic layer was extracted using DCM (3*50 mL). The organic layer was dried with sodium sulfate, filtered and concentrated in the rotavapor.

To purify the crude a chromatography column (5*12 cm) was used and as solvent hexane and 5% EtOAc/Hex were used.

This reaction was done more three times one using 3,3 grams of **4** and the other using 42 grams of **4**.

Aspect: Yellow oil

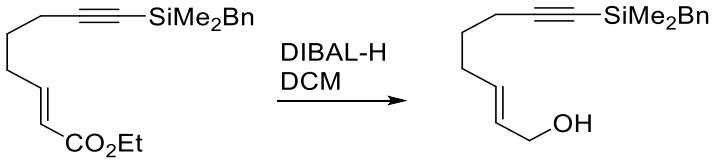
R_F = 0,80 (20% AcOEt/Hex)

ɳ = 84%

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 – 7.04 (5H, m, H_{Ph}), 6.94 (1H, m, H-3), 5.84 (1H, dt, *J* = 15.6, 1.5 Hz, H-2), 4.20 (2H, q, *J* = 7.1 Hz, CO_2CH_2), 2.29 (4H, m, H-4 and H-6), 2.18 (2H, s, CH_2Si), 1.67 (2H, m, H-5), 1.30 (3H, t, *J* = 7.1 Hz,$CO_2CH_2C\underline{H_3}$), 0.11 (6H, s, Si-Me_2).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.69 (C, C-1), 148.10 (CH, C-3), 139.32 (C, C_{Ph}), 128.48 (2*CH, C_{Ph}), 128.24 (2*CH, C_{Ph}), 124.42 (CH, C_{Ph}), 122.17 (CH, C-2), 107.83 (C, C-8), 83.93 (C, C-7), 60.34 (CH_2, CO_2CH_2), 31.11 (CH_2), 26.91 (CH_2), 26.59 (CH_2), 19.43 (CH_2), 14.41 (CH_3, $CO_2CH_2CH_3$), -1.78 (2*CH_3, 2CH_3-Si).).

3.1.5. Synthesis of (E)-8-(benzyldimethylsilyl)oct-2-en-7-yn-1-ol (**12**)



11 **12**

The compound **11** was dried and putted under argon. Then DCM (≈350 mL) was added through a cannula. The solution was then cooled in an ice bath and left stirring for a little while. Then a solution of DIBAL-H in Hexane (153 mL, 153,4 mmol, 2eq.) was slowly added. The mixture was transferred to a separation funnel and a saturated solution of NaCl and some drops of HCl 5% were added. Was formed a huge emulsion, very hard to undo. The organic layer was extracted with DCM, then was dried with sodium sulfate, filtered and concentrated in the rotavapor.

To purify the crude a chromatography column of SiO_2 (5*12) was used and was used as eluent first 5% EtOAc/Hex and then 10% EtOAc/Hex.

Aspect: Yellow oil

R_F = 0,80 (20% AcOEt/Hex)

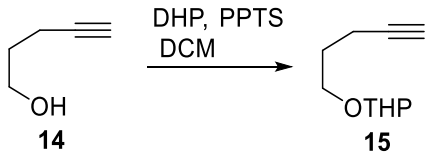
η = 54%

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 – 7.04 (5H, m, H_{Ph}), 5.76 – 5.60 (2H, m, H-2 and H-3), 4.19 – 4.01 (2H, m, H-1), 2.24 (2H, t, *J* = 7.1 Hz, H-6), 2.18 (2H, s, CH_2Si), 2.17 – 2.12 (2H, m, H-4), 1.65 – 1.56 (2H, m, H-5), 1.43 (1H, s, OH), 0.11 (6H, s, Si-Me_2).

^{13}C NMR (101 MHz, $CDCl_3$) δ 139.40 (C, C_{Ph}), 132.15 (CH, C-2), 130.00 (CH,C-3), 128.51 (2*CH, C_{Ph}), 128.24 (2*CH, C_{Ph}), 124.39 (CH,C_{Ph}), 108.59 (C, C-8), 83.43 (C, C-7), 63.85 (CH_2-C-1), 31.27 (CH_2), 28.02 (CH_2), 26.66 (CH_2), 19.42 (CH_2), -1.76 (2*CH_3, 2CH_3-Si).

3.2. Bicyclic ring 5,5,

3.2.1. Synthesis of Synthesis of 2-(pent-4-yn-1-yloxy) tetrahydro-2H-pyran (**15**)



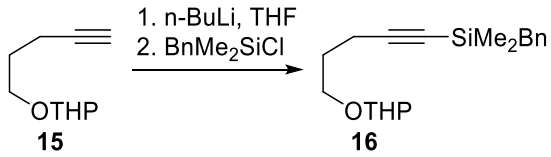
To a 250 mL round bottom flask compound **14** (20g, 238 mmol, 1 eq.), dried DCM (≈200 mL), PPTS (3g, 11,89 mmol, 0,05 eq.) and DHP (30g, 357mmol,1,5eq.) were added. This mixture was left to react for 3 days. The mixture was concentrated in the rotavapor and purified in a SiO_2 column (5*10 cm). As eluent was used 10% EtOAc/Hex.

This same experience was repeated with 18 grams of **14**.

Aspect: Yellow oil

R_F = 0,70 (20% AcOEt/Hex)

3.2.2. Synthesis of benzyldimethyl(5-((tetrahydro-2H-pyran-2-yl) oxy)pent-1-yn-1-yl) silane (**16**)



Using a cannula compound **15** (33,9 g, 202 mmol, 1 eq.) was transferred to a 500 mL round bottom flask, already containing Et_2O (200 mL). The mixture was cooled and then was slowly added the n-BuLi (79 mL, 197 mmol, 1,1 eq.). This reaction was left stirring 1 hour out of the cold bath. The mixture was cooled again at 50 °C and the $ClSiMe_2Bn$ (40,97 g, 221mmol, 1.1 eq). was added. The mixture was taken from the bath and left stirring for 24 hours.

To stop the reaction was added water (10mL). The mixture was concentrated in the rotavapor and transferred to a separation funnel and the organic layer was extracted with Et_2O (150 mL). The organic layer was dried with sodium sulfate, filtered and then concentrated in the rotavapor.

To purify the crude was used a chromatography column of SiO_2, (5*14cm), and was used as eluent hexane, 5% EtOAc/Hex and lastly 10 % EtOAc/Hex.

This reaction was done again but using 38,7 grams of **15** compound.

Aspect: Yellow oil

References

1. Norman, A. W., The History of the Discovery of Vitamin D and Its Daughter Steroid Hormone. *Annals of Nutrition and Metabolism* **2012,** *61* (3), 199-206.
2. Ihde, A. J., Studies on the History of Rickets. II : The Roles of Cod Liver Oil and Light. *Pharmacy in History* **1975,** *17* (1), 13-20.
3. Huldschinsky, K., Heilung von Rachitis durch künstliche Höhensonne. *Dtsch med Wochenschr* **1919,** *45* (26), 712-713.
4. Hess, A. F.; Unger, L. J., The cure of infantile rickets by artificial light and by sunlight. *Proceedings of the Society for Experimental Biology and Medicine* **1921,** *18* (8), 298-298.
5. Jarrett, P.; Scragg, R., A short history of phototherapy, vitamin D and skin disease. *Photochemical & Photobiological Sciences* **2017,** *16* (3), 283-290.
6. Askew, F. A.; Bourdillon, R. B.; Bruce, H. M.; Jenkins, R. G. C.; Webster, T. A.; Dale, H. H., The distillation of vitamin D. *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character* **1930,** *107* (748), 76-90.
7. Norman, A. W., CHAPTER 3 - The Vitamins D. In *Vitamin D*, Norman, A. W., Ed. Academic Press: 1979; pp 37-69.
8. DeLuca, H. F., Chapter One - Vitamin D: Historical Overview. In *Vitamins & Hormones*, Litwack, G., Ed. Academic Press: 2016; Vol. 100, pp 1-20.
9. Windaus, A.; Bock, F., *Über das Provitamin aus dem Sterin der Schweineschwarte*. 2019; Vol. 245, p 168-174.
10. Esvelt, R. P.; Schnoes, H. K.; DeLuca, H. F., Vitamin D3 from rat skins irradiated in vitro with ultraviolet light. *Archives of Biochemistry and Biophysics* **1978,** *188* (2), 282-286.
11. Holick, M.; MacLaughlin, J.; Clark, M.; Holick, S.; Potts, J.; Anderson, R.; Blank, I.; Parrish, J.; Elias, P., Photosynthesis of previtamin D3 in human skin and the physiologic consequences. *Science* **1980,** *210* (4466), 203-205.
12. Knudson, A.; Benford, F., Quantitative studies of the effectiveness of ultraviolet radiation of various wave-lengths in rickets. *Journal of Biological Chemistry* **1938,** *124*, 287-299.
13. Wacker, M.; Holick, M. F., Vitamin D - effects on skeletal and extraskeletal health and the need for supplementation. *Nutrients* **2013,** *5* (1), 111-148.
14. Holick, M. F., Resurrection of vitamin D deficiency and rickets. *J Clin Invest* **2006,** *116* (8), 2062-2072.
15. Alshahrani, F.; Aljohani, N., Vitamin D: Deficiency, Sufficiency and Toxicity. *Nutrients* **2013,** *5* (9).
16. Omdahl, J. L.; Morris, H. A.; May, B. K., HYDROXYLASE ENZYMES OF THE VITAMIN D PATHWAY: Expression, Function, and Regulation. *Annual Review of Nutrition* **2002,** *22* (1), 139-166.
17. Jones, G.; Prosser, D. E.; Kaufmann, M., 25-Hydroxyvitamin D-24-hydroxylase (CYP24A1): Its important role in the degradation of vitamin D. *Archives of Biochemistry and Biophysics* **2012,** *523* (1), 9-18.
18. Henry, H. L., Vitamin D hydroxylases. *Journal of Cellular Biochemistry* **1992,** *49* (1), 4-9.
19. Bouillon, R., Chapter 5 - The Vitamin D Binding Protein DBP. In *Vitamin D (Third Edition)*, Feldman, D.; Pike, J. W.; Adams, J. S., Eds. Academic Press: San Diego, 2011; pp 57-72.
20. Mangelsdorf, D. J.; Thummel, C.; Beato, M.; Herrlich, P.; Schütz, G.; Umesono, K.; Blumberg, B.; Kastner, P.; Mark, M.; Chambon, P.; Evans, R. M., The nuclear receptor superfamily: The second decade. *Cell* **1995,** *83* (6), 835-839.
21. Pike, J. W.; Meyer, M. B.; Lee, S. M., Chapter 7 - The Vitamin D Receptor: Biochemical, Molecular, Biological, and Genomic Era Investigations. In *Vitamin D (Third Edition)*, Feldman, D.; Pike, J. W.; Adams, J. S., Eds. Academic Press: San Diego, 2011; pp 97-135.

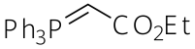
22. DeLuca, H. F., Overview of general physiologic features and functions of vitamin D. *The American Journal of Clinical Nutrition* **2004,** *80* (6), 1689S-1696S.
23. Rosen, C. J.; Adams, J. S.; Bikle, D. D.; Black, D. M.; Demay, M. B.; Manson, J. E.; Murad, M. H.; Kovacs, C. S., The Nonskeletal Effects of Vitamin D: An Endocrine Society Scientific Statement. *Endocrine Reviews* **2012,** *33* (3), 456-492.
24. Rochel, N.; Wurtz, J. M.; Mitschler, A.; Klaholz, B.; Moras, D., The Crystal Structure of the Nuclear Receptor for Vitamin D Bound to Its Natural Ligand. *Molecular Cell* **2000,** *5* (1), 173-179.
25. Rochel, N.; Moras, D., Chapter 9 - Structural Basis for Ligand Activity in VDR. In *Vitamin D (Third Edition)*, Feldman, D.; Pike, J. W.; Adams, J. S., Eds. Academic Press: San Diego, 2011; pp 171-191.
26. Mizwicki, M. T.; Keidel, D.; Bula, C. M.; Bishop, J. E.; Zanello, L. P.; Wurtz, J.-M.; Moras, D.; Norman, A. W., Identification of an alternative ligand-binding pocket in the nuclear vitamin D receptor and its functional importance in 1α,25(OH)₂-vitamin D₃ signaling. *Proc Natl Acad Sci U S A* **2004,** *101* (35), 12876-12881.
27. Norman, A. W.; Mizwicki, M. T.; Norman, D. P. G., Steroid-hormone rapid actions, membrane receptors and a conformational ensemble model. *Nature Reviews Drug Discovery* **2004,** *3* (1), 27-41.
28. Haussler, M. R.; Jurutka, P. W.; Mizwicki, M.; Norman, A. W., Vitamin D receptor (VDR)-mediated actions of 1α,25(OH)2vitamin D3: Genomic and non-genomic mechanisms. *Best Practice & Research Clinical Endocrinology & Metabolism* **2011,** *25* (4), 543-559.
29. Plum, L. A.; DeLuca, H. F., Vitamin D, disease and therapeutic opportunities. *Nature Reviews Drug Discovery* **2010,** *9*, 941.
30. Dusso, A. S.; Brown, A. J., Chapter 19 - Vitamin D: Molecular Biology and Gene Regulation. In *Textbook of Nephro-Endocrinology (Second Edition)*, Singh, A. K.; Williams, G. H., Eds. Academic Press: 2018; pp 347-371.
31. JONES, G.; STRUGNELL, S. A.; DeLUCA, H. F., Current Understanding of the Molecular Actions of Vitamin D. *Physiological Reviews* **1998,** *78* (4), 1193-1231.
32. Verlinden, L.; Eelen, G.; Bouillon, R.; Vandewalle, M.; De Clercq, P.; Verstuyf, A., Chapter 76 - Analogs of Calcitriol. In *Vitamin D (Third Edition)*, Feldman, D.; Pike, J. W.; Adams, J. S., Eds. Academic Press: San Diego, 2011; pp 1461-1487.
33. Sicinska, W.; Westler, W. M.; DeLuca, H. F., NMR assignments of tryptophan residue in apo and holo LBD-rVDR. *Proteins: Structure, Function, and Bioinformatics* **2005,** *61* (3), 461-467.
34. DeLuca, H. F.; Plum, L. A., Chapter 74 - Alterations in 1,25-Dihydroxyvitamin D3 Structure that Produce Profound Changes in in Vivo Activity. In *Vitamin D (Third Edition)*, Feldman, D.; Pike, J. W.; Adams, J. S., Eds. Academic Press: San Diego, 2011; pp 1429-1435.
35. Posner, Gary H.; Kahraman, M., Organic Chemistry of Vitamin D Analogues (Deltanoids). *European Journal of Organic Chemistry* **2003,** *2003* (20), 3889-3895.
36. Stein, M. S.; Wark, J. D., An update on the therapeutic potential of vitamin D analogues. *Expert Opinion on Investigational Drugs* **2003,** *12* (5), 825-840.
37. Bronson, J.; Dhar, M.; Ewing, W.; Lonberg, N., Chapter Thirty-One - To Market, To Market—2011. In *Annual Reports in Medicinal Chemistry*, Desai, M. C., Ed. Academic Press: 2012; Vol. 47, pp 499-569.
38. Ringe, J. D.; Schacht, E., Improving the outcome of established therapies for osteoporosis by adding the active D-hormone analog alfacalcidol. *Rheumatology International* **2007,** *28* (2), 103-111.
39. Dheerendra, P. C.; Sakhuja, V.; Kohli, H. S.; Jha, V., Efficacy and safety of oral doxercalciferol in the management of secondary hyperparathyroidism in chronic kidney disease stage 4. *Indian J Nephrol* **2013,** *23* (4), 271-275.
40. Nishii, Y.; Okano, T., History of the development of new vitamin D analogs: studies on 22-oxacalcitriol (OCT) and 2β-(3-hydroxypropoxy)calcitriol (ED-71). *Steroids* **2001,** *66* (3), 137-146.

41. Glebocka, A.; Chiellini, G., A-ring analogs of 1,25-dihydroxyvitamin D3. *Archives of Biochemistry and Biophysics* **2012,** *523* (1), 48-57.
42. Dai, H.; Posner, G. H., Synthetic Approaches to Vitamin D. *Synthesis* **1994,** *1994* (12), 1383-1398.
43. Baggiolini, E. G.; Iacobelli, J. A.; Hennessy, B. M.; Batcho, A. D.; Sereno, J. F.; Uskokovic, M. R., Stereocontrolled total synthesis of 1.alpha.,25-dihydroxycholecalciferol and 1.alpha.,25-dihydroxyergocalciferol. *The Journal of Organic Chemistry* **1986,** *51* (16), 3098-3108.
44. Zhu, G.-D.; Okamura, W. H., Synthesis of Vitamin D (Calciferol). *Chemical Reviews* **1995,** *95* (6), 1877-1952.
45. Dawson, T. M.; Dixon, J.; Littlewood, P. S.; Lythgoe, B.; Saksena, A. K., Calciferol and its relatives. Part XVI. Total synthesis of precalciferol32. *Journal of the Chemical Society C: Organic* **1971,** (0), 2960-2966.
46. Mascareñas, J. L.; Sarandeses, L. A.; Castedo, L.; Mouriño, A., Palladium-catalysed coupling of vinyl triflates with enynes and its application to the synthesis of 1α,25-dihydroxyvitamin D3. *Tetrahedron* **1991,** *47* (20), 3485-3498.
47. Aurrecoechea, J. M.; Okamura, W., A short, enantiospecific synthesis of the 1α-hydroxyvitamin D enyne A-ring synthon. *Tetrahedron Letters* **1987,** *28*, 4947–4950.
48. Trost, B. M.; Dumas, J.; Villa, M., New strategies for the synthesis of vitamin D metabolites via palladium-catalyzed reactions. *Journal of the American Chemical Society* **1992,** *114* (25), 9836-9845.
49. Gogoi, P.; Sigüeiro, R.; Eduardo, S.; Mouriño, A., An Expeditious Route to 1α,25-Dihydroxyvitamin D3 and Its Analogues by an Aqueous Tandem Palladium-Catalyzed A-Ring Closure and Suzuki Coupling to the C/D Unit. *Chemistry – A European Journal* **2010,** *16* (5), 1432-1435.
50. Mouriño, A.; Torneiro, M.; Vitale, C.; Fernández, S.; Pérez-Sestelo, J.; Anné, S.; Gregorio, C., Efficient and versatile synthesis of a-ring precursors of 1α,25-dihydroxy-vitamin D3 and analogues. Application to the synthesis of Lythgoe-Roche phosphine oxide. *Tetrahedron Letters* **1997,** *38* (26), 4713-4716.
51. Pandey, G.; Raikar, S. B., A simple strategy for the synthesis of optically pure trans-hydrindane systems. *Tetrahedron Letters* **2006,** *47* (12), 2029-2032.
52. Baggiolini, E. G.; Iacobelli, J. A.; Hennessy, B. M.; Uskokovic, M. R., Stereoselective total synthesis of 1.alpha.,25-dihydroxycholecalciferol. *Journal of the American Chemical Society* **1982,** *104* (10), 2945-2948.
53. Micheli, R. A.; Hojos, Z. G.; Cohen, N.; Parrish, D. R.; Portland, L. A.; Sciamanna, W.; Scott, M. A.; Wehrli, P. A., Total syntheses of optically active 19-nor steroids. (+)-Estr-4-ene-3,17-dione and (+)-13.beta.-ethylgon-4-ene-3,17-dione. *The Journal of Organic Chemistry* **1975,** *40* (6), 675-681.
54. Chapleo, C. B.; Hallett, P.; Lythgoe, B.; Waterhouse, I.; Wright, P. W., Calciferol and its relatives. Part 19. Synthetic applications of cyclic orthoesters: stereospecific synthesis of a bicyclic alcohol related to the vitamins D2. *Journal of the Chemical Society, Perkin Transactions 1* **1977,** (10), 1211-1218.
55. López-Pérez, B.; Maestro, M. A.; Mouriño, A., Total synthesis of 1α,25-dihydroxyvitamin D3 (calcitriol) through a Si-assisted allylic substitution. *Chemical Communications* **2017,** *53* (58), 8144-8147.
56. Wishart, D. S., Improving Early Drug Discovery through ADME Modelling. *Drugs in R & D* **2007,** *8* (6), 349-362.
57. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J., Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **1997,** *23* (1–3), 3.
58. Vrbanac, J.; Slauter, R., Chapter 3 - ADME in Drug Discovery. In *A Comprehensive Guide to Toxicology in Nonclinical Drug Development (Second Edition)*, Faqi, A. S., Ed. Academic Press: Boston, 2017; pp 39-67.

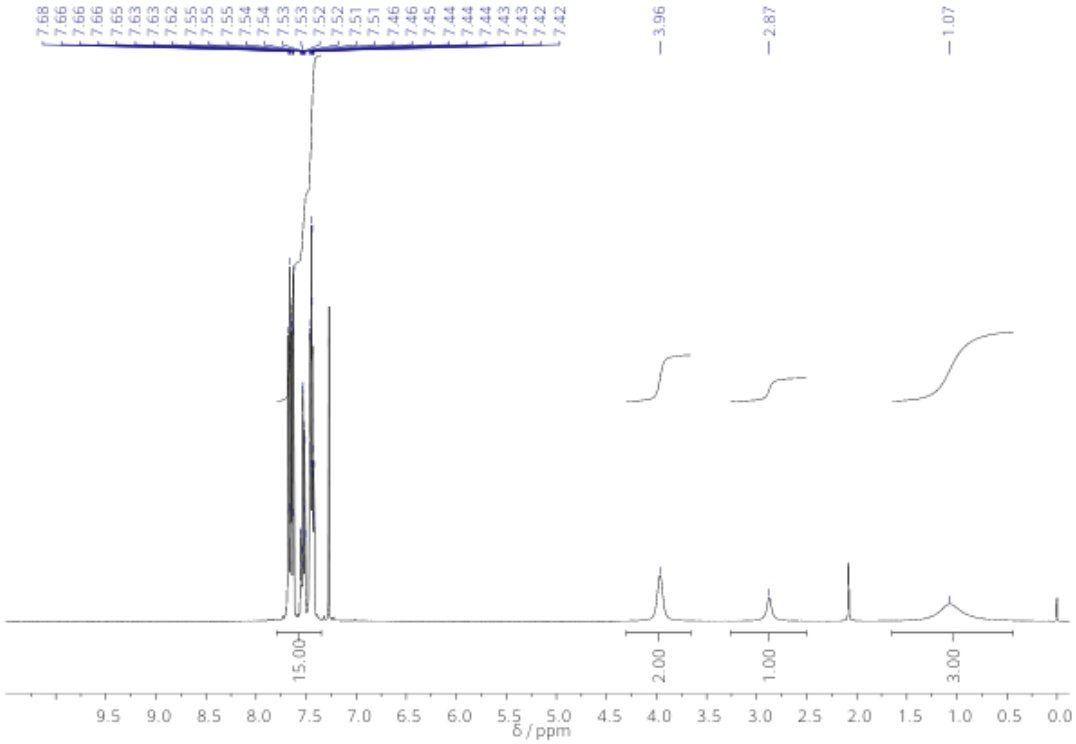
59. Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R., Development and validation of a genetic algorithm for flexible docking11Edited by F. E. Cohen. *Journal of Molecular Biology* **1997,** *267* (3), 727-748.
60. Berdullas, P. G. Diseño y exploración sintética de análogos inéditos de la Vitamina D modificados en el biciclo CD. Universidade de Santiago de Compostela, 2018.
61. Magalhães, N. Síntese de percursores avançados de novoa anaálogos da 1α,25-dihidroxi vitamina D_3 modificados na cadeia lateral. Faculadade de Ciências da Universidade do Porto, 2016.
62. Gallenkamp, D.; Fürstner, A., Stereoselective Synthesis of E,Z-Configured 1,3-Dienes by Ring-Closing Metathesis. Application to the Total Synthesis of Lactimidomycin. *Journal of the American Chemical Society* **2011,** *133* (24), 9232-9235.
63. Corey, E. J.; Suggs, J. W., Pyridinium chlorochromate. An efficient reagent for oxidation of primary and secondary alcohols to carbonyl compounds. *Tetrahedron Letters* **1975,** *16* (31), 2647-2650.
64. Adrio, J.; Rodríguez Rivero, M.; Carretero, J. C., endo-Selective Intramolecular Pauson–Khand Reactions of γ-Oxygenated-α,β-unsaturated Phenylsulfones. *Chemistry – A European Journal* **2001,** *7* (11), 2435-2448.
65. Corey, E. J.; Venkateswarlu, A., Protection of hydroxyl groups as tert-butyldimethylsilyl derivatives. *Journal of the American Chemical Society* **1972,** *94* (17), 6190-6191.
66. Lima, B. L. P. d. Síntese de percurssores quirais de sila - Derivados da vitamina D 2019.
67. Khand, I. U.; Knox, G. R.; Pauson, P. L.; Watts, W. E.; Foreman, M. I., Organocobalt complexes. Part II. Reaction of acetylenehexacarbonyldicobalt complexes, (R1C2R2)Co2(CO)6, with norbornene and its derivatives. *Journal of the Chemical Society, Perkin Transactions 1* **1973,** (0), 977-981.
68. Czakó, L. K. a. B., Pauson-Khand reaction. In *Strategic Applications of Named Reactions in Organic Synthesis*, Elsevier: 2005; pp 334-335.
69. Magnus, P.; Principe, L. M., Origins of 1,2- and 1,3-stereoselectivity in dicobaltoctacarbonyl alkene-alkyne cyclizations for the synthesis of substituted bicyclo[3.3.0]octenones. *Tetrahedron Letters* **1985,** *26* (40), 4851-4854.
70. Blanco-Urgoiti, J.; Añorbe, L.; Pérez-Serrano, L.; Domínguez, G.; Pérez-Castells, J., The Pauson–Khand reaction, a powerful synthetic tool for the synthesis of complex molecules. *Chemical Society Reviews* **2004,** *33* (1), 32-42.
71. Raminelli, C.; V. Comasseto, J.; H. Andrade, L.; L. M. Porto, A., Kinetic Resolution of Propargylic and Allylic Alcohols by Candida antarctica Lipase (Novozyme 435). *Cheminform* **2005,** *36*.
72. Novozym® 435. Strem Chemicals, I., Ed.
73. Jing, Q.; Kazlauskas, R. J., Determination of absolute configuration of secondary alcohols using lipase-catalyzed kinetic resolutions. *Chirality* **2008,** *20* (5), 724-735.
74. Martin, V. S.; Woodard, S. S.; Katsuki, T.; Yamada, Y.; Ikeda, M.; Sharpless, K. B., Kinetic resolution of racemic allylic alcohols by enantioselective epoxidation. A route to substances of absolute enantiomeric purity? *Journal of the American Chemical Society* **1981,** *103* (20), 6237-6240.
75. Czakó, L. K. a. B., Sharpless asymmetric epoxidation. In *Strategic Applications of Named Reactions in Organic Synthesis*, Elsevier: 2005; pp 408-409.
76. Woodard, S. S.; Finn, M. G.; Sharpless, K. B., Mechanism of asymmetric epoxidation. 1. Kinetics. *Journal of the American Chemical Society* **1991,** *113* (1), 106-113.
77. De Mico, A.; Margarita, R.; Parlanti, L.; Vescovi, A.; Piancatelli, G., A Versatile and Highly Selective Hypervalent Iodine (III)/2,2,6,6-Tetramethyl-1-piperidinyloxyl-Mediated Oxidation of Alcohols to Carbonyl Compounds. *The Journal of Organic Chemistry* **1997,** *62* (20), 6974-6977.
78. Iwabuchi, Y., Discovery and Exploitation of AZADO: The Highly Active Catalyst for Alcohol Oxidation. *Chemical and Pharmaceutical Bulletin* **2013,** *61* (12), 1197-1213.
79. Czakó, L. K. a. B., Wittig reaction. In *Strategic applications of named reactions in organic synthesis*, Elsevier: pp 486-487.

80. Maryanoff, B. E.; Reitz, A. B., The Wittig olefination reaction and modifications involving phosphoryl-stabilized carbanions. Stereochemistry, mechanism, and selected synthetic aspects. *Chemical Reviews* **1989,** *89* (4), 863-927.
81. Gagey, N.; Neveu, P.; Benbrahim, C.; Goetz, B.; Aujard, I.; Baudin, J.-B.; Jullien, L., Two-Photon Uncaging with Fluorescence Reporting: Evaluation of the o-Hydroxycinnamic Platform. *Journal of the American Chemical Society* **2007,** *129* (32), 9986-9998.

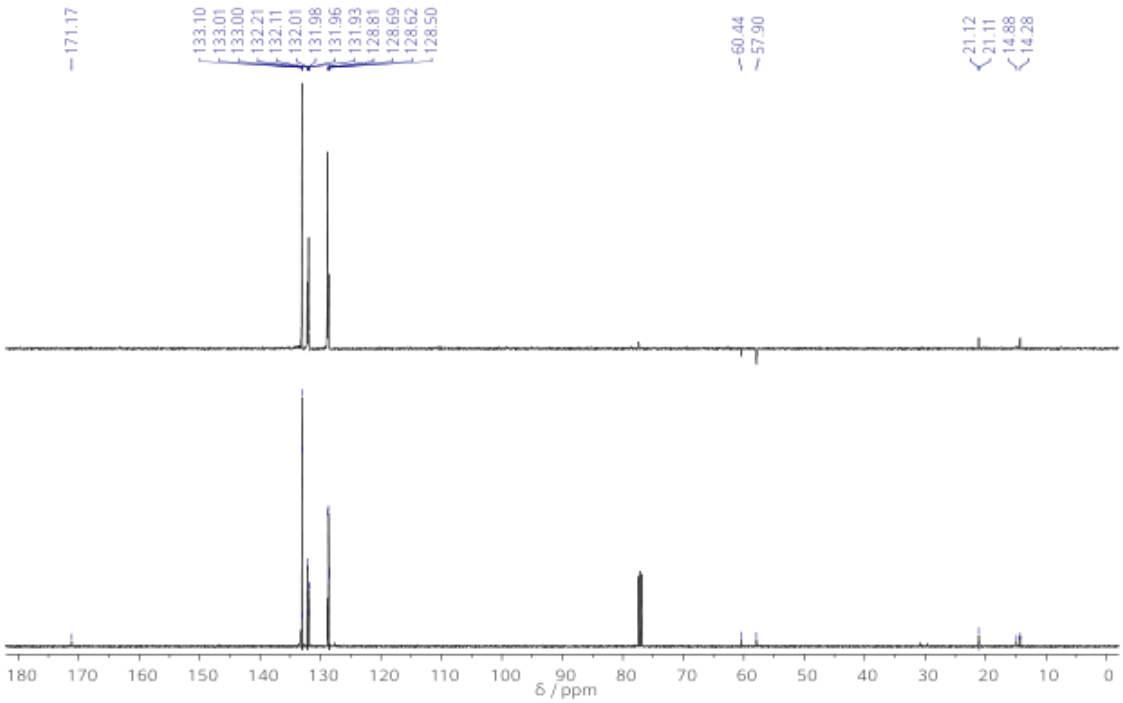
Appendix

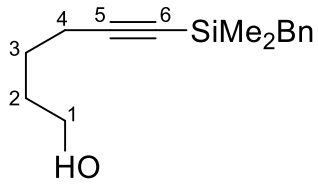


^{1}H NMR (400 MHz, CDCl3) δ 7.98 – 7.11 (15H, m, H_{ph}), 3.96 (s, 2H, H_{Et}), 2.87 (1H, s, $H_{double\ bond}$), 1.07 (3H, s, H_{Et}).



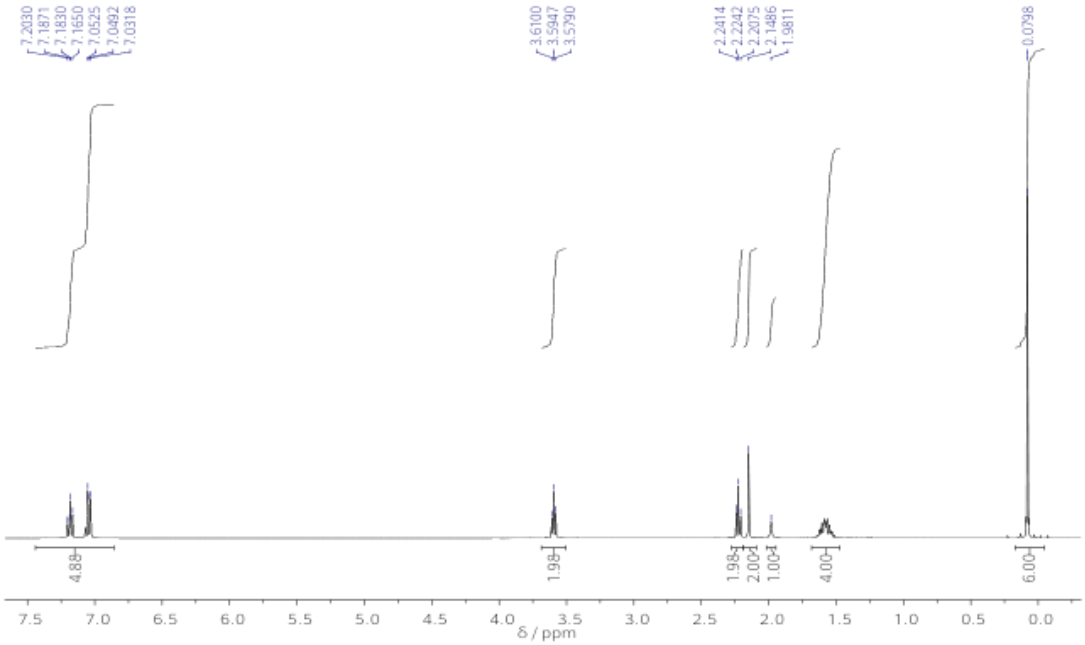
^{13}C NMR (101 MHz, $CDCl_3$) δ 171.17 (C, C=O), 133.20 – 128.66 (C_{Ph}), [60.44 (CH_2), 57.90 (CH_2), C_{Et}], [21.12 (CH), 21.11 (CH), $C_{doublebond}$], [14.88 (CH_3), 14.28 (CH_3), C_{Et}]



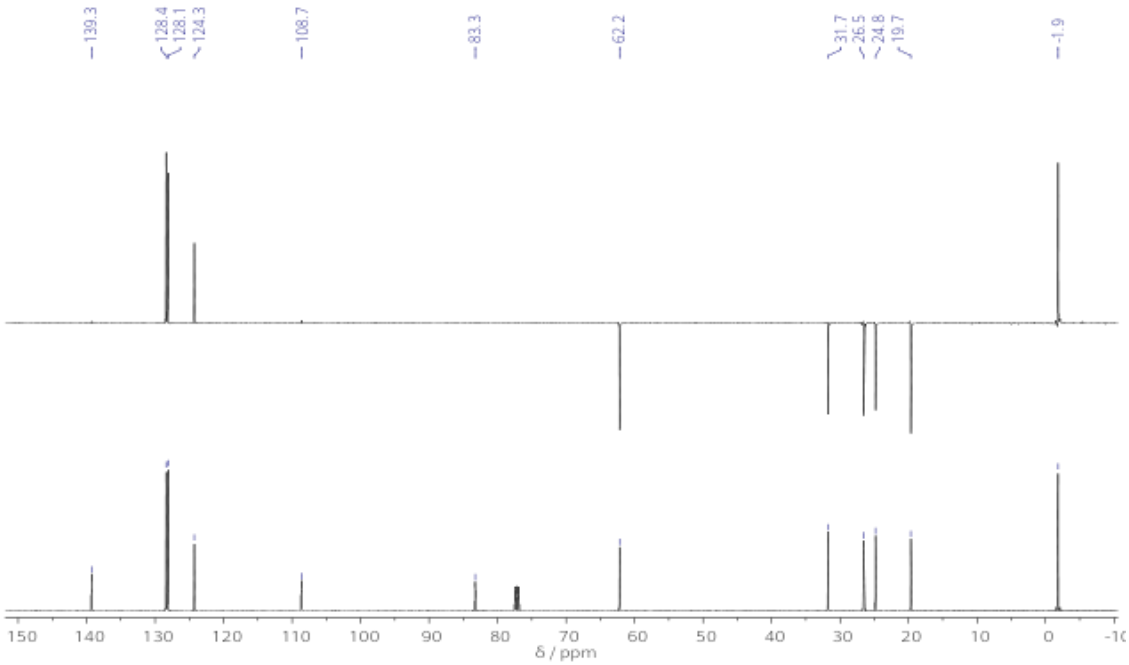


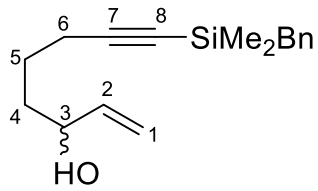
4

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 – 7.03 (5H, m, H_{Ph}), 3.59 (2H, t, *J* = 6.2 Hz, H-1), 2.22 (2H, t, *J* = 6.8 Hz, H-4), 2.15 (2H, s, CH_2Si), 1.98 (1H, s, OH), 1.68 – 1.47 (4H, m, C-3 and C-4), 0.08 (6H, s, Si-Me_2).



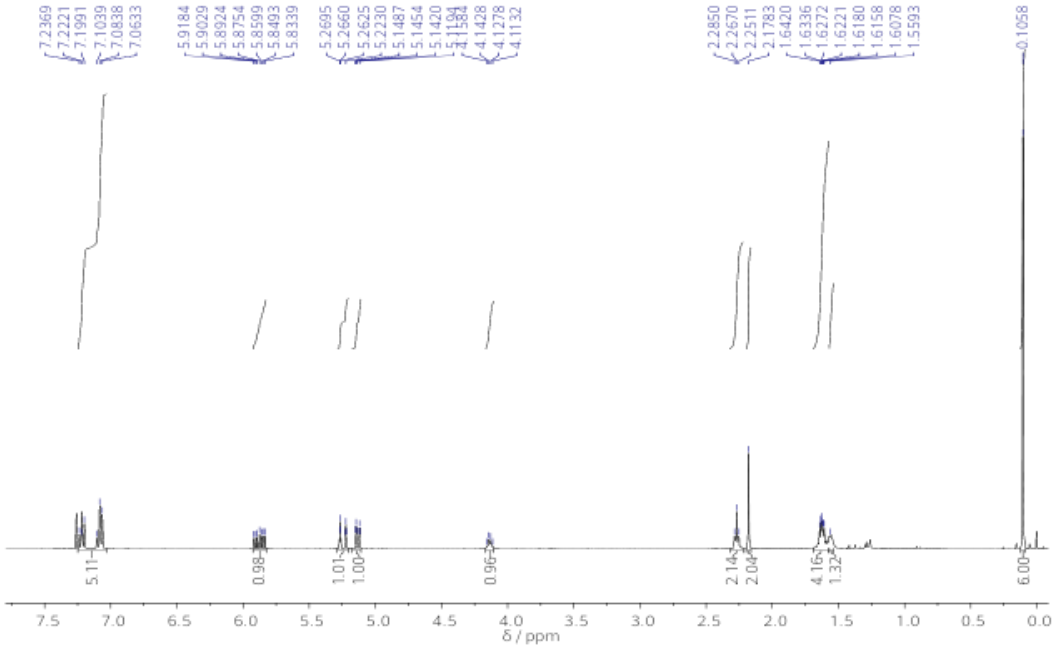
^{13}C NMR (101 MHz, $CDCl_3$) δ 139.27 (C, C_{Ph}), 128.40 (2*CH, C_{Ph}), 128.13 (2*CH, C_{Ph}), 124.28 (CH, C_{Ph}), 108.65 (C, C-6), 83.26 (C, C-5), 62.20 (CH_2, C-1), 31.73 (CH_2), 26.52 (CH_2), 24.84 (CH_2), 19.66 (CH_2), -1.85 (2*CH_3, 2CH_3-Si).



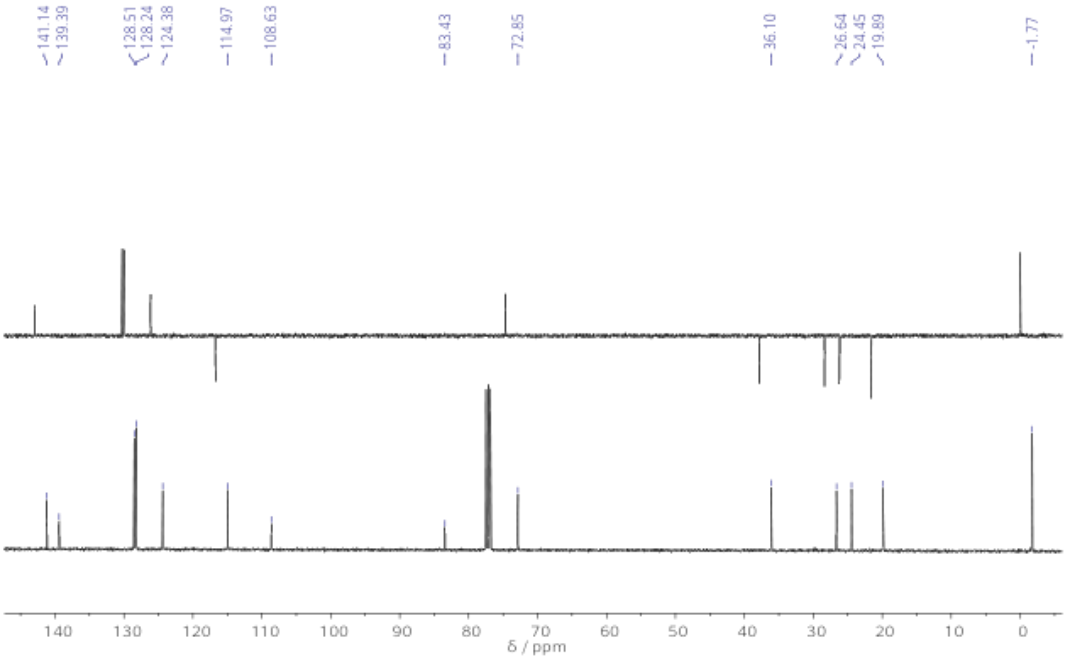


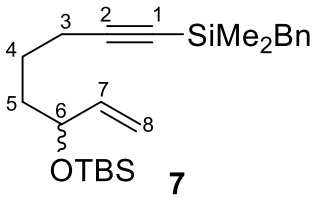
6

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 – 7.04 (5H, m, H_{Ph}), 5.93 – 5.82 (1H, m, H-2), 5.24 (1H, dt, *J* = 17.2, 1.4 Hz, H-1), 5.13 (1H, dt, *J* = 10.4, 1.3 Hz, H-1), 4.14 (1H, q, *J* = 6.0 Hz, H-3), 2.27 (2H, t, *J* = 6.8 Hz, H-6), 2.18 (2H, s, CH_2Si), 1.69 – 1.58 (4H, m,H-4 and H-5), 1.56 (1H, s,OH), 0.11 (6H,s, Si-Me_2).



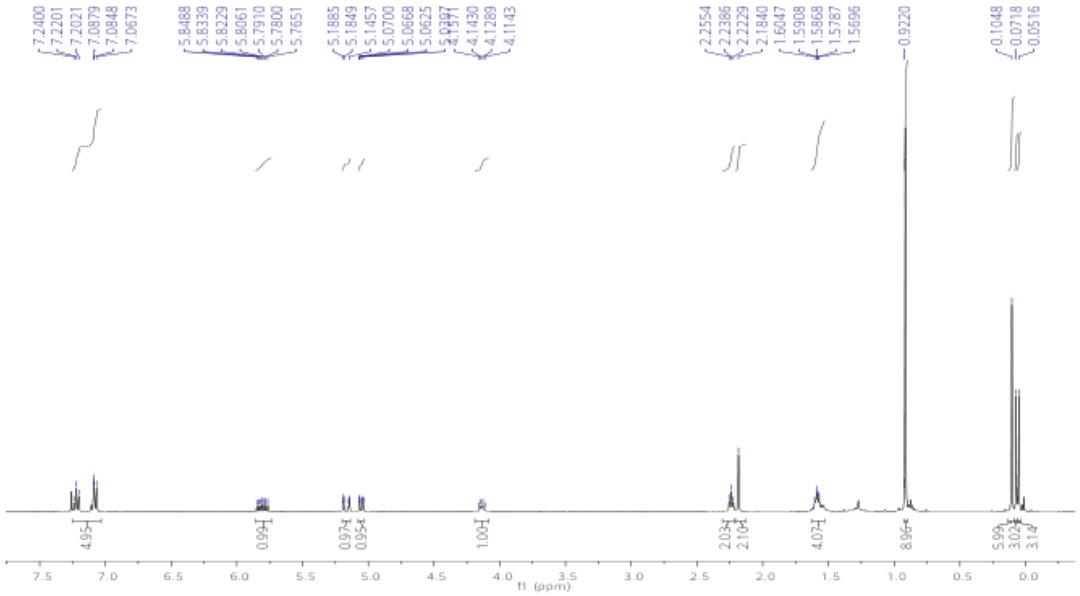
^{13}C NMR (101 MHz, $CDCl_3$) δ 141.14 (CH, C-2), 139.39 (C,C_{Ph}), 128.51 (2*CH, C_{Ph}), 128.24 (2*CH,C_{Ph}), 124.38 (CH, C_{Ph}), 114.97 (CH_2, C-1), 108.63 (C, C-8), 83.43 (C, C-7), 72.85 (CH, C-3), 36.10 (CH_2), 26.64 (CH_2), 24.45 (CH_2), 19.89 (CH_2), -1.77 (2*CH_3, 2CH_3-Si).



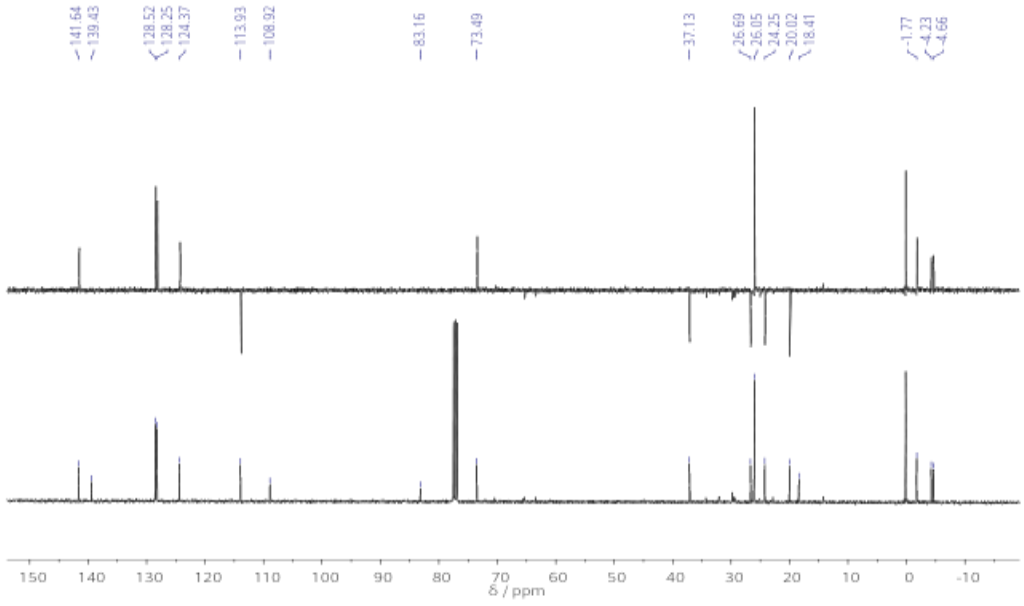


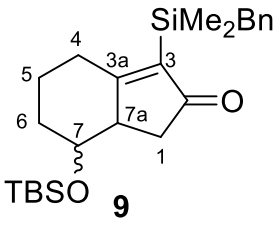
7

^{1}H NMR (400 MHz, CDCl3) δ 7.25 – 7.03 (5H, m, H_{Ph}), 5.86 – 5.75 (1H, m, H-7), 5.20 – 5.14 (1H, m, H-8), 5.08 – 5.03 (1H, m, H-8), 4.14 (1H, q, *J* = 5.7 Hz, H-6), 2.24 (2H, t, J = 6.5 Hz, H-3), 2.18 (2H, s, CH_2Si), 1.69 – 1.50 (4H,m, H-4 and H-5), 0.92 (9H,s, *t*-Bu), 0.10 (6H, s, Si-Me_2), 0.07 (3H, s, CH_3-Si), 0.05 (3H, s, CH_3-Si).



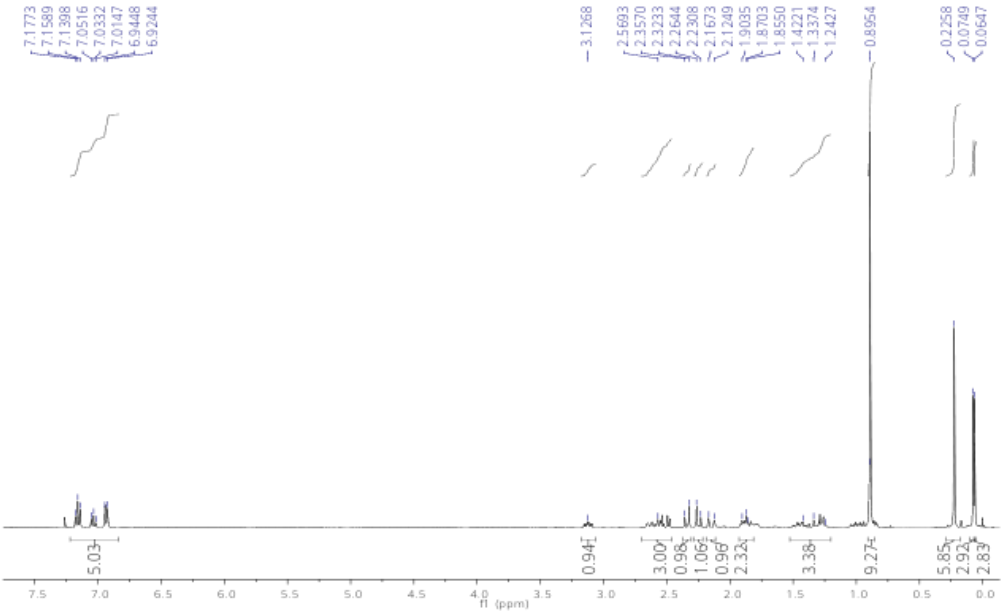
^{13}C NMR (101 MHz, $CDCl_3$) δ 141.64 (CH, C-7), 139.43 (C, C_{Ph}), 128.52 (2*CH, C_{Ph}), 128.25 (2*CH, C_{Ph}), 124.37 (CH, C_{Ph}), 113.93 (CH_2, C-8), 108.92 (C, C-1), 83.16 (C, C-2), 73.49 (CH, C-6), 37.13 (CH_2), 26.69 (CH_2), 26.05 (3*CH_3, *t*-Bu), 24.25 (CH_2), 20.02 (CH_2), 18.41 (C, C-Si), -1.77 (2*CH_3, Si-Me_2), -4.23 (CH_3, CH_3-Si), -4.66 (CH_3, CH_3-Si).



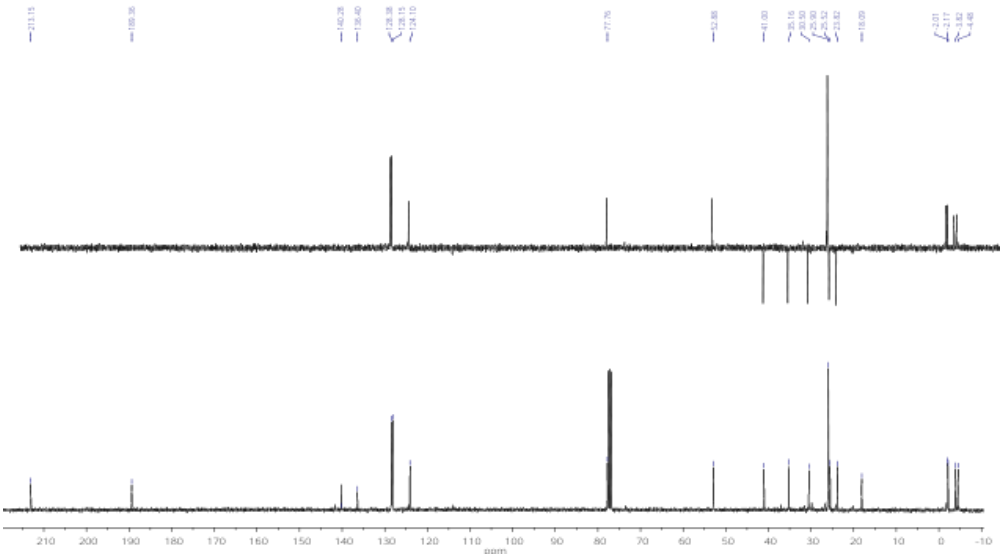


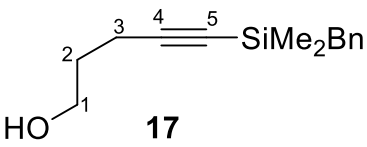
9

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 – 6.85 (5H, m,H_{Ph}), 3.19 – 3.07 (1H, m, H-7), 2.70 – 2.47 (3H, m), 2.34 (1H,d, *J* = 13.5 Hz, CH_2Si), 2.25 (1H, d, *J* = 13.4 Hz, CH_2Si), 2.15 (1H, d, *J* = 16.9 Hz, H-7a), 1.88 (2H, m, H-1), 1.36 (3H,m), 0.90 (9H,s, *t*-Bu), 0.23 (6H, s, Si-Me_2), 0.07 (3H,s, CH_3-Si), 0.06 (3H, s, CH_3-Si).



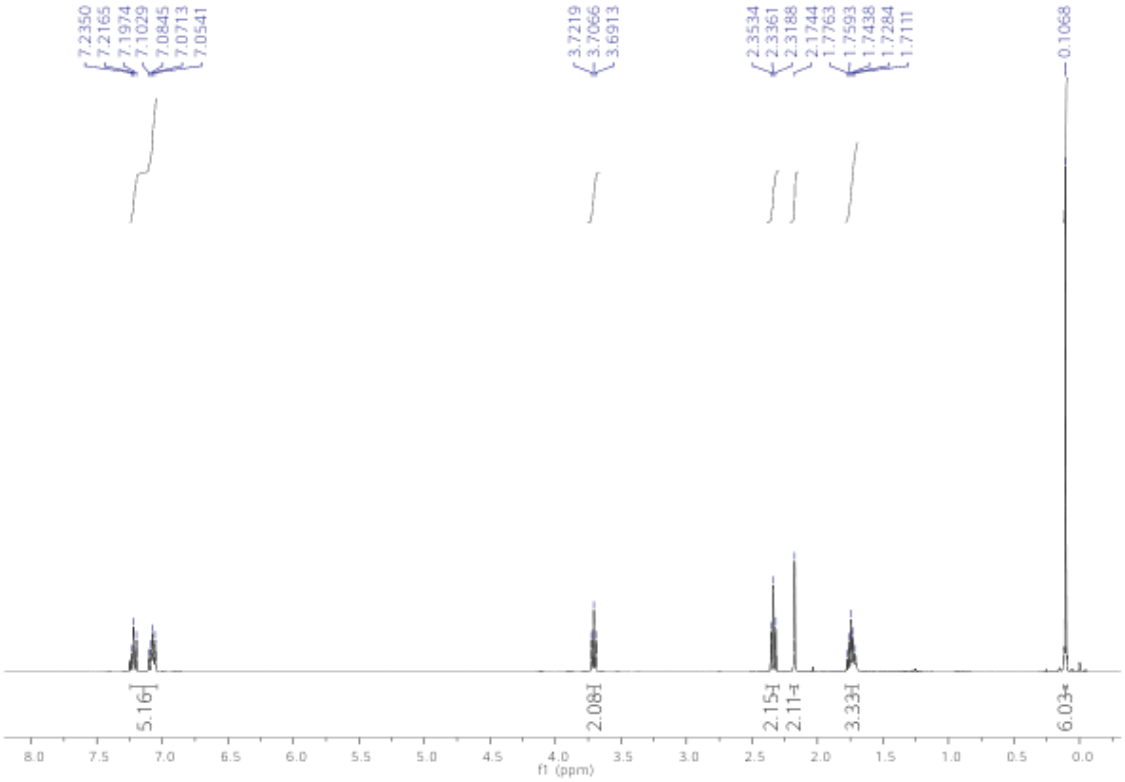
^{13}C NMR (101 MHz, $CDCl_3$) δ 213.1 (C, C-2), 189.4 (C, C-3a), 140.3 (C, C_{Ph}), 136.4 (C, C-3), 128.38 (2*CH_2, C_{Ph}) 128.15 (2*CH_2, C_{Ph}), 124.10 (CH, C_{Ph}), 77.76 (CH, C-7), 52.88(CH, C-7a) 41.00 (CH_2), 35.16(CH_2), 30.5(CH_2), 25.90 (3*CH_3, *t*-Bu), 25.52 (CH_2), 23.82 (CH_2), 18.09 (C, *t*-Bu), -2.01 (CH3, CH_3-Si), -2.17 (CH3, CH_3-Si), -3.82 (CH3, CH_3-Si), -4.48 (CH3, CH_3-Si)

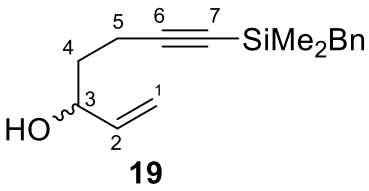




17

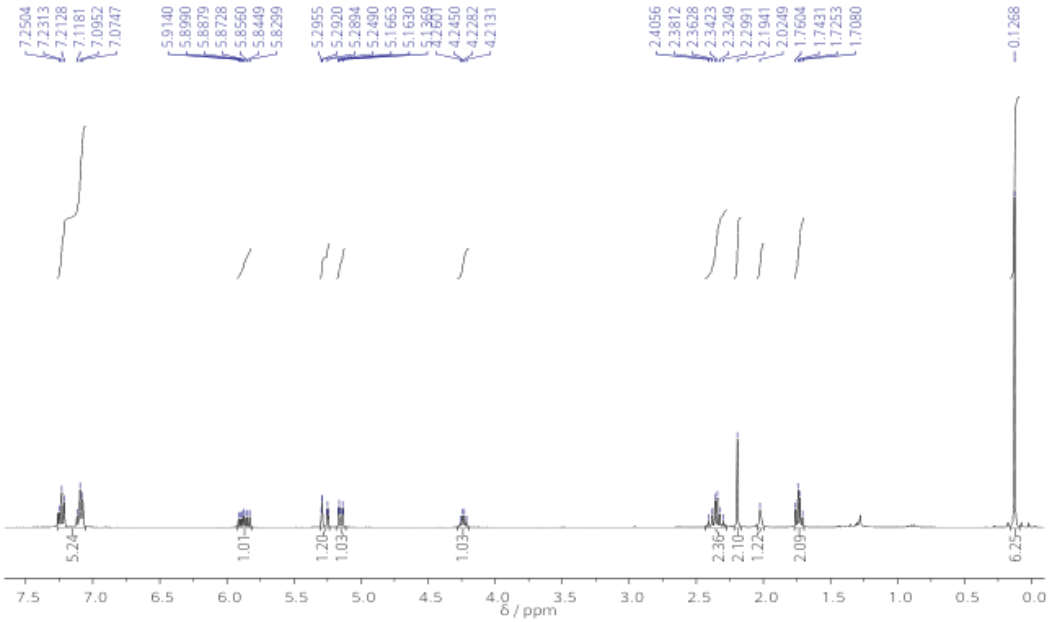
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 – 7.04 (5H, m, H_{Ph}), 3.71 (2H, t, *J* = 6.1 Hz, H-1), 2.34 (2H, t, *J* = 6.9 Hz, H-3), 2.17 (2H ,s, CH_2Si), 1.81 – 1.66 (3H, m, H-2 and OH), 0.11 (6H, s, Si-Me_2).



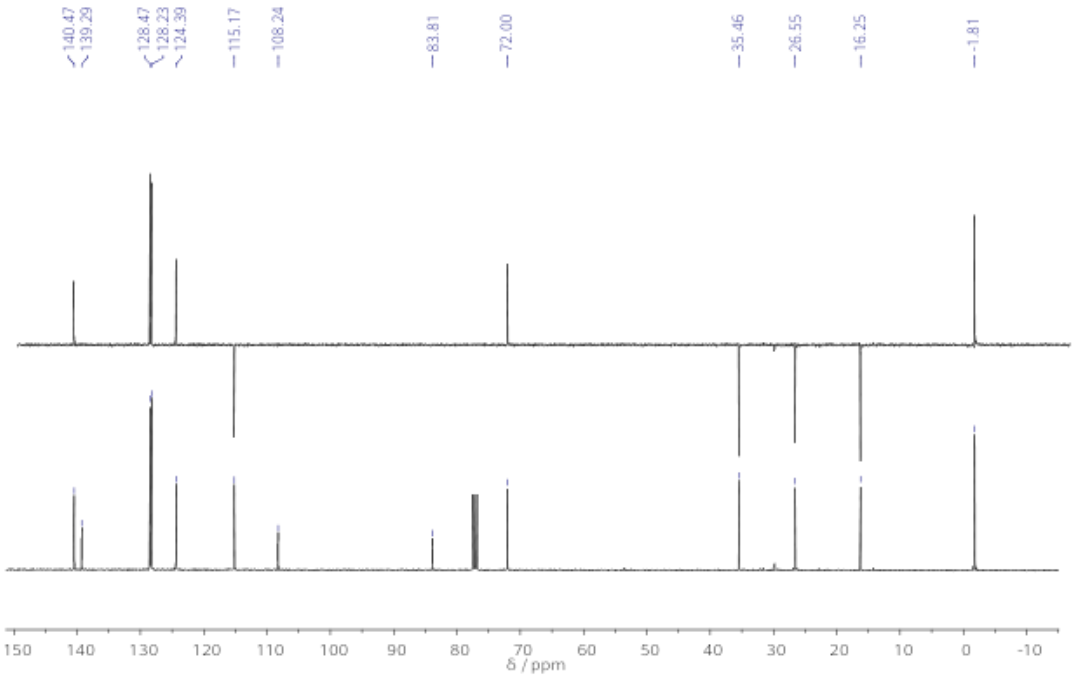


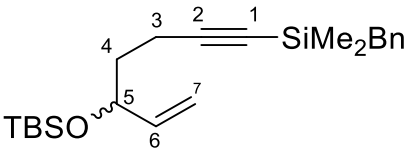
19

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (5H, m, H_{Ph}), 5.94 – 5.78 (1H, m, H-2), 5.27 (1H, dt, *J* = 17.6, 1.4 Hz, H-1), 5.15 (1H, dt, *J* = 10.4, 1.3 Hz, H-1), 4.24 (1H, dd, *J* = 12.7, 6.0 Hz, H-3), 2.44 – 2.28 (2H, m, H-5), 2.19 (2H, s, CH_2Si), 2.02 (1H ,s, OH), 1.73 (2H, dd, *J* = 14.1, 6.9 Hz, H-4), 0.13 (6H, s, Si-Me_2)



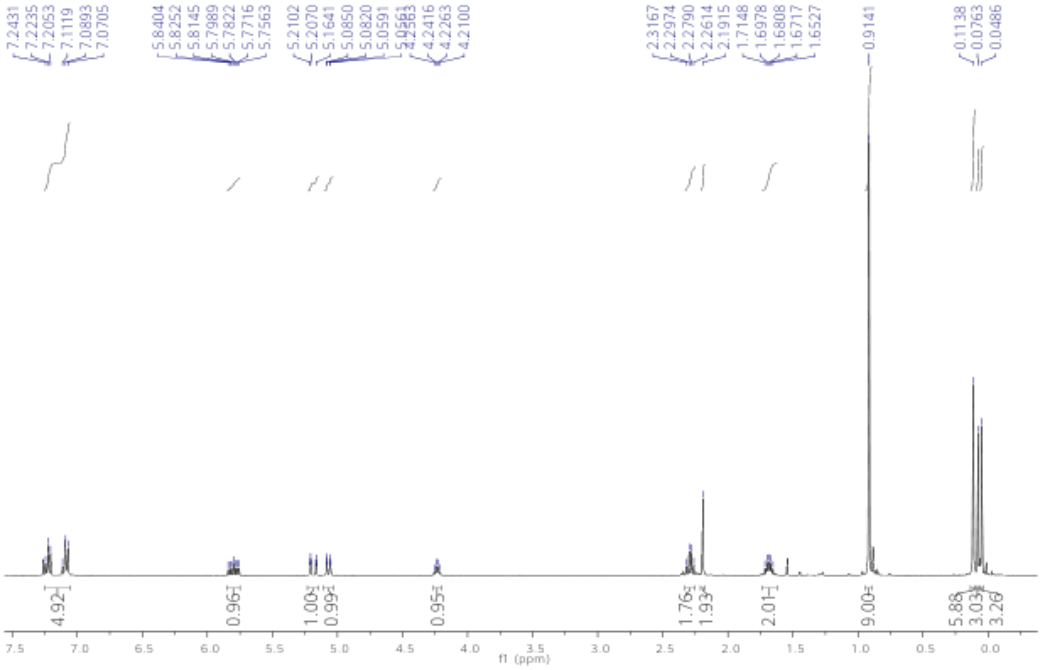
^{13}C NMR (101 MHz, $CDCl_3$) δ 140.47 (CH, C-2), 139.29 (C, C_{Ph}), 128.47 (2*CH, C_{Ph}), 128.23 (2*CH, C_{Ph}), 124.39 (CH, C_{Ph}), 115.17 (CH_2, C-1), 108.24 (C, C-7), 83.81 (C, C-6), 72.00 (CH, C-3), 35.46 (CH_2), 26.55 (CH_2), 16.25 (CH_2), -1.81 (2*CH_3, 2CH_3-Si).



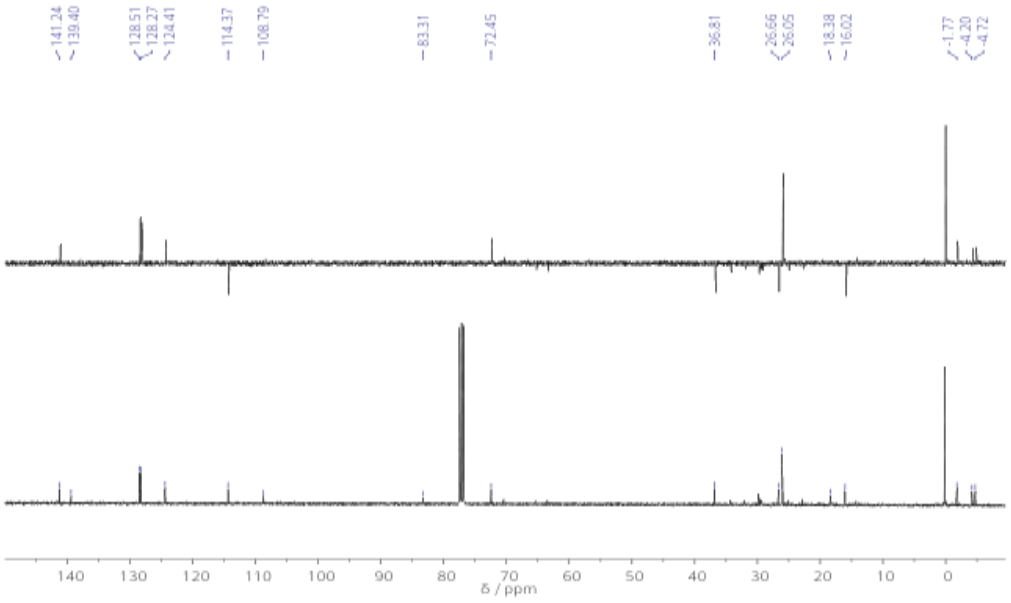


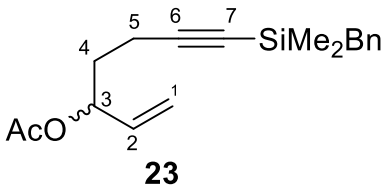
20

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 – 7.07 (5H,m, H_{Ph}), 5.86 – 5.74 (1H, m, H-6), 5.13 (2H, m, H-7), 4.23 (1H, dd, *J* = 12.3, 6.2 Hz, H-5), 2.29 (2H, dd, *J* = 14.7, 7.4 Hz, H-3), 2.19 (2H , s, CH_2Si), 1.77 – 1.63 (2H, m, H-4), 0.91 (9H, s, *t*-Bu), 0.11 (6H, s, Si-Me_2), 0.08 (3H, s, CH_3-Si), 0.05 (3H, s, CH_3-Si).



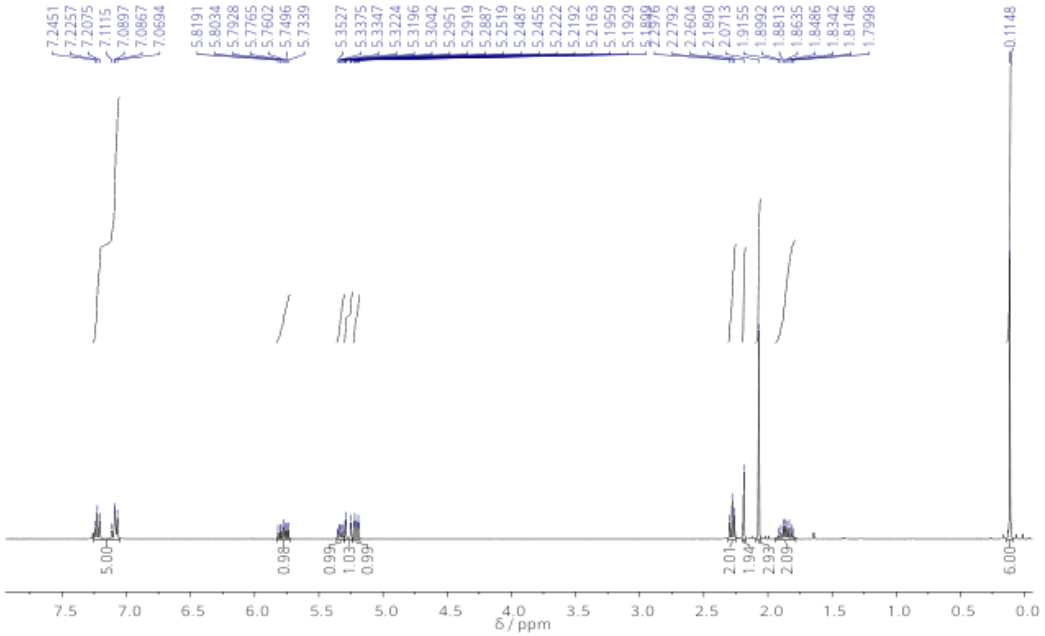
^{13}C NMR (101 MHz, $CDCl_3$) δ 141.24 (CH, C-6), 139.40 (C, C_{Ph}), 128.51 (2*CH, C_{Ph}), 128.27 (2*CH, C_{Ph}), 124.41 (CH, C_{Ph}), 114.37 (CH_2, C-7), 108.79 (C, C-1), 83.31 (C, C-2), 72.45 (CH, C-5), 36.81 (CH_2), 26.66 (CH_2), 26.05 (3*CH_3, *t*-Bu), 18.38 (C, C-Si), 16.02 (CH_2), -1.77 (2*CH_3, Si-Me_2), -4.20 (CH_3, CH_3-Si), -4.72 (CH_3, CH_3-Si).



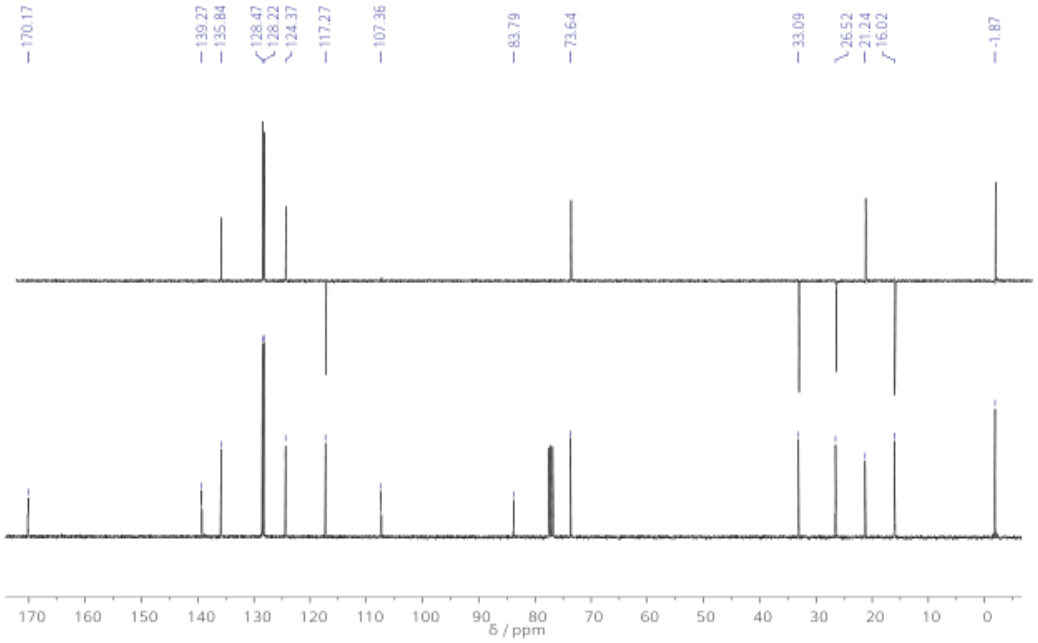


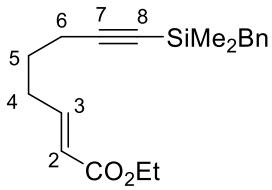
23

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 – 7.02 (5H, m, H_{Ph}), 5.78 (1H, m, H-2), 5.36 – 5.30 (1H, m, H-3), 5.27 (1H, dt, *J* = 17.3, 1.3 Hz, H-1), 5.21 (1H, dt, *J* = 10.5, 1.2 Hz, H-1), 2.28 (2H, t, *J* = 7.4 Hz, H-5), 2.19 (2H, s, CH_2Si), 2.07 (3H, s, H_{AcO}), 1.86 (2H, m, H-4), 0.11 (6H, s, Si-Me_2).



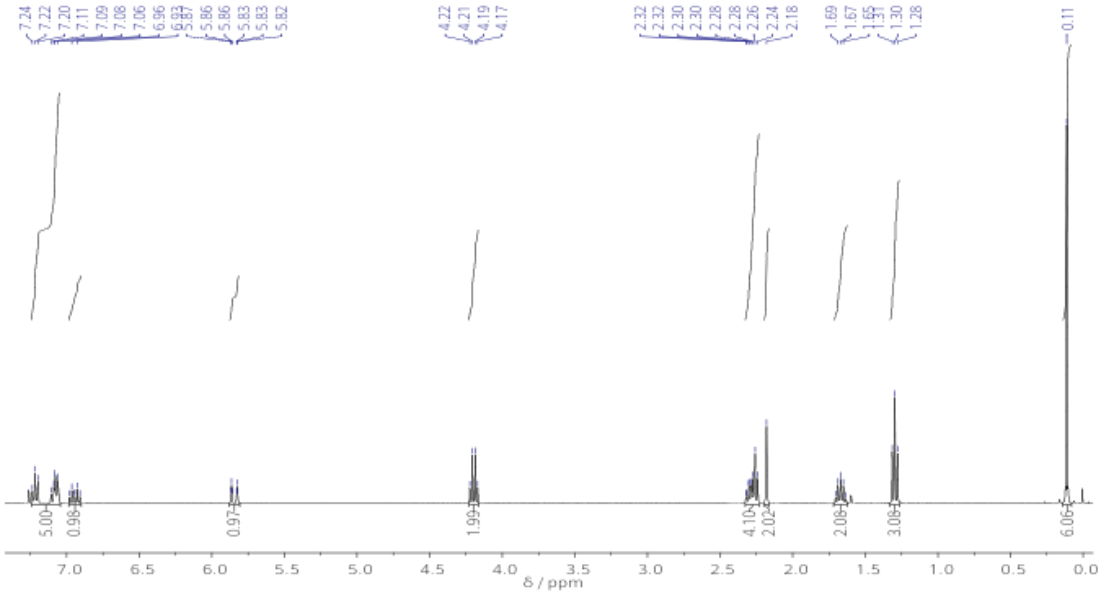
^{13}C NMR (101 MHz, $CDCl_3$) δ 170.17 (C, C=O), 139.27 (C, C_{Ph}), 135.84 (CH, C-2), 128.47 (2*CH, C_{Ph}), 128.22 (2*CH, C_{Ph}), 124.37 (CH, C_{Ph}), 117.27 (CH_2, C-1), 107.36 (C, C-7), 83.79 (C, C-6), 73.64 (CH, C-3), 33.09 (CH_2), 26.52 (CH_2), 21.24 (CH_3,AcO), 16.02 (CH_2), -1.87 (2*CH_3, 2CH_3-Si).



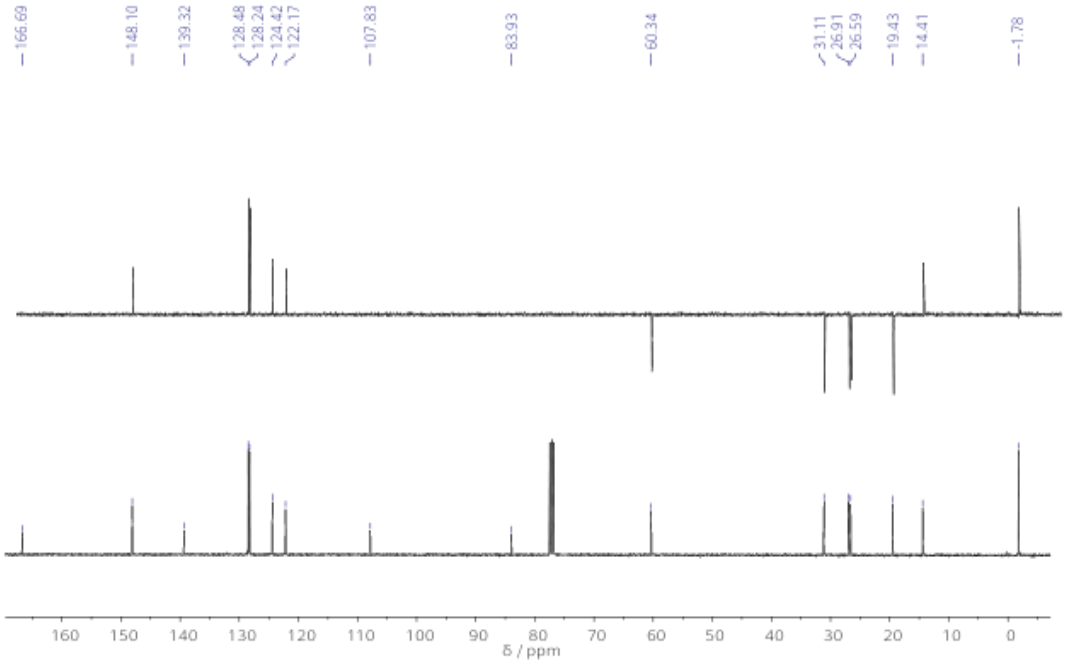


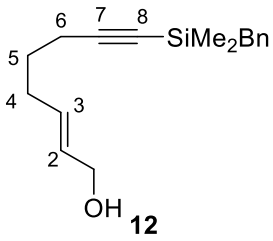
11

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 – 7.04 (5H, m, H_{Ph}), 6.94 (1H, m, H-3), 5.84 (1H, dt, *J* = 15.6, 1.5 Hz, H-2), 4.20 (2H, q, *J* = 7.1 Hz, CO_2CH_2), 2.29 (4H, m, H-4 and H-6), 2.18 (2H, s, CH_2Si), 1.67 (2H, m, H-5), 1.30 (3H, t, *J* = 7.1 Hz,$CO_2CH_2C\underline{H_3}$), 0.11 (6H, s, Si-Me_2).



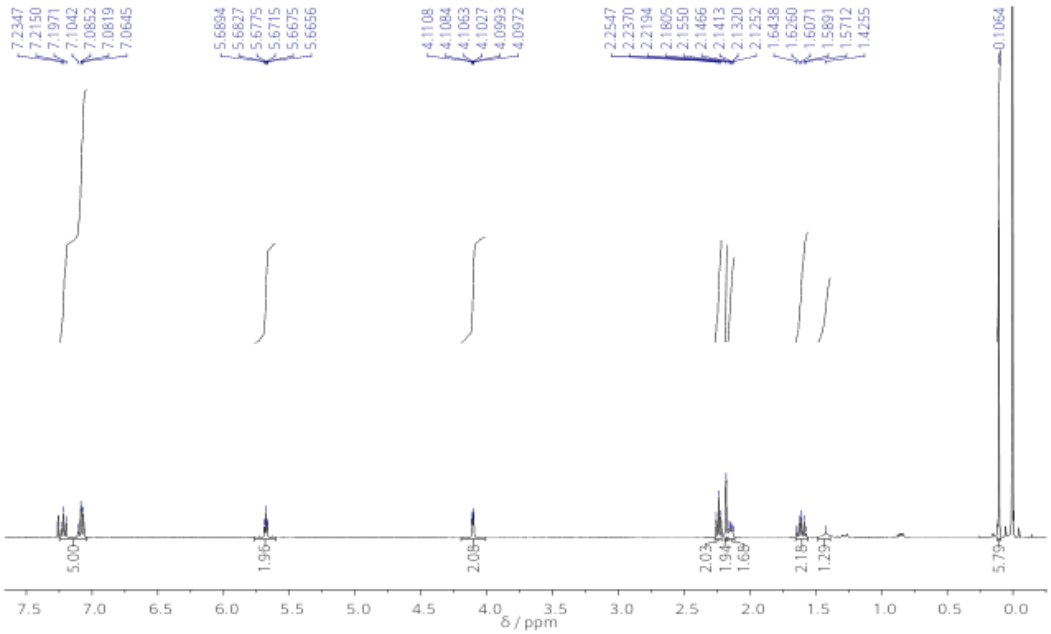
^{13}C NMR (101 MHz, $CDCl_3$) δ 166.69 (C, C-1), 148.10 (CH, C-3), 139.32 (C, C_{Ph}), 128.48 (2*CH, C_{Ph}), 128.24 (2*CH, C_{Ph}), 124.42 (CH, C_{Ph}), 122.17 (CH, C-2), 107.83 (C, C-8), 83.93 (C, C-7), 60.34 (CH_2, CO_2CH_2), 31.11 (CH_2), 26.91 (CH_2), 26.59 (CH_2), 19.43 (CH_2), 14.41 (CH_3, $CO_2CH_2CH_3$), -1.78 (2*CH_3, 2CH_3-Si).).





12

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 – 7.04 (5H, m, H_{Ph}), 5.76 – 5.60 (2H, m, H-2 and H-3), 4.19 – 4.01 (2H, m, H-1), 2.24 (2H, t, *J* = 7.1 Hz, H-6), 2.18 (2H, s, CH_2Si), 2.17 – 2.12 (2H, m, H-4), 1.65 – 1.56 (2H, m, H-5), 1.43 (1H, s, OH), 0.11 (6H, s, Si-Me_2).



^{13}C NMR (101 MHz, $CDCl_3$) δ 139.40 (C, C_{Ph}), 132.15 (CH, C-2), 130.00 (CH,C-3), 128.51 (2*CH, C_{Ph}), 128.24 (2*CH, C_{Ph}), 124.39 (CH,C_{Ph}), 108.59 (C, C-8), 83.43 (C, C-7), 63.85 (CH_2-C-1), 31.27 (CH_2), 28.02 (CH_2), 26.66 (CH_2), 19.42 (CH_2), -1.76 (2*CH_3, 2CH_3-Si).